Peer Review File

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Reviewer A

This study is a post-hoc analysis of outcomes in the CT arm of the LUSI lung cancer screening study where subjects underwent baseline spirometry. This study is somewhat underpowered but reports important differences in over-all and lung cancer specific mortality according to the presence of airflow limitation or restrictive spirometry. This study confirms the results of other studies (see ref 22) showing that about one third of screening subjects had abnormal lung function, the majority of which had no prior diagnosis. This study also confirms past studies showing that airflow limitation is associated with a histology shift (see ref 47) and increased deaths from non-lung cancer causes (see ref 33).

The following needs addressing.

Comment 1. The data in all tables would be better presented in portrait not landscape.

Reply 1: We agree. Actually, the text files that we submitted (also those of the tables) were in portrait format, not landscape. When we received the reviewers' comments, we ourselves noted that comments referred to page and line numbers that did not correspond to those in our submitted files. Upon further inquiry, it appeared that the TLCR editorial office had reformatted manuscript and tables before submitting these to the reviewers. Upon our request, we have now received the reformatted manuscript.

Changes in text: none

Comment 2. The data in Tables 2, 3 and 5, where COPD has been divided into GOLD stage groups is just too small to be statistically reliable despite apparently significant p values. I don't think this stratification by GOLD stage should be presented due to small numbers and I think conclusions from these should be omitted or drastically tempered.

Reply 2: Thank you. Given a similar suggestion also from reviewer 2, we have moved some of the results on GOLD-stage subgroups (Tables 2, 3) to the online supplemental materials, and otherwise (Table 5) deleted these altogether; see below (changes in text).

Changes made to text: The results on GOLD stage groups originally in Tables 2 and 3 have been moved to the Supplement (new Supplemental Table 1), and those originally in Table 5 have been deleted altogether. Furthermore, we have now removed any findings pertinent to COPD subgroups from the Abstract and main conclusions section (Discussion) of our manuscript.

Comment 3. In Supplementary table 1a the N= 280 not 1725. In Tables 4 and 6 p values should be included.

Changes made to text:

- Supplementary Table 1a: Thank you very much for spotting this typo – this has been corrected
- P-values for Tables 4 and 6: We have added these, as requested

Comment 4. In the discussion the authors need to emphasise that the prevalence of both COPD and Prism in the screening population of high risk smokers is significantly less that that found in the lung cancer group. While not surprising, and consistent with many other studies, is completely over-looked in lung cancer case-control studies reporting biomarker associations where confounding by these two unrecognised phenotypes is otherwise ignored.

Reply 4. The fact that prevalence of COPD and PRISm in the lung cancer group was (even) higher than that in the overall screening population of long-term smokers is highlighted by the fact that there was an approximately 2- to 2.5-fold estimated increase in lung cancer risk (odds ratios) associated with moderate-to-severe COPD or PRISm, which we emphasized very much in our discussion and abstract.
However, we have added a sentence to underline that this relative risk (odds ratio) does indeed reflect a higher prevalence of COPD and PRISm in the lung cancer case group.

Changes in text. We added the following further sentence to the Discussion: "*Overall, it is worth highlighting that 55 per cent of the lung cancer cases observed in the LUSI screening arm had either COPD or PRISm according to the baseline spirometry examination*."

Comment 5. On line 94 in the introduction and 420 in the discussion there is a full colon that should be referenced.

Change in text: Thank you. In both instances, to avoid confusion, the sentences following the colon should have started with small capital: we have now corrected this.

Comment 6. On lines 454 and 467 in the discussion please replace "Study" with "Trial".

Change in text: Thank you; we have now corrected this.

Comment 7. On line 454 the NLST-ARCIN study should be included as a study where spirometry was included.

Change made in text. Thank you; we added this to line 454.

Reviewer B

In this manuscript, the authors examine the association between lung function categories (normal, COPD, PRISm) assessed at baseline and prospective all-cause mortality and lung cancer risk within the intervention arm of the German Lung Cancer Screening Study (LUSI). Findings include an increased risk of all-cause mortality and lung cancer among both COPD and PRISm groups relative to normal spirometry and, among those with lung cancer, evidence supporting diagnosis at more advanced stages and non-adenocarcinoma histological subtypes among both COPD and PRISm groups. Strengths include a relatively large sample size, use of spirometry, and objective, validated endpoints (mortality, lung cancer diagnosis). Relative weaknesses include the use of inconsistent or non-standard terminology, failure to adjust for additional potential confounders in mortality analyses, and incomplete discussion of limitations and prior publications in the field. Specific comments are as follows:

Major Comments:

Comment 1. Use of consistent / standard terminology - In pulmonology, "airflow limitation" is typically used in reference to obstructive lung disease. Recommend using "lung function impairment" or "abnormal spirometry" instead of "airflow limitation" or "airflow impairment" throughout the manuscript when referring to both COPD and PRISm groups together. Similarly, would recommend using "PRISm" throughout instead of alternating between "PRISm" and "restrictive spirometry". Please consider changing title + running title to "Lung function impairment in the German Lung Cancer Screening Study (LUSI)...." for consistency.

Reply 1: We thank the reviewer for these helpful clarifications regarding terminology.

Changes in text: Throughout the text, wherever relevant, we have replaced “airflow imitation” by the terms “lung function impairment” or “abnormal spirometry”, and “restrictive spirometry” by “PRISm”. In addition, we changed the title of the manuscript, as suggested.

Comment 2. Abstract - Please define LUSI abbreviation, enrollment criteria besides age (e.g. cumulative smoking requirements, time since quitting requirements), and spirometry cutoffs for lung function categories. This will be critical for casual readers to understand the study.

Changes in text: To the abstract we added the full name of the LUSI study, as well as the criteria (spirometry cutoffs) for lung function categories (COPD, PRISm, normal). Unfortunately, space restrictions (Abstract word limit) does not allow the full (rather lengthy) description of the enrollment criteria; we therefore kept the wording “*50-69 year-old long-term smokers*”, without the extensive further specification.

Comment 3. Introduction - (p3, line 91) - chronic bronchitis and emphysema are historical subtypes of COPD, not causes of COPD - please modify this sentence. (p3, lines 100-101) - obstructive and restrictive diseases can co-exist in the same person, but only when full lung function (total lung capacity) is assessed. When only spirometry is available (as in this study), obstruction and PRISm are mutually exclusive categories. The reference cited (10 - Kinney et al) refers to radiographic emphysema versus airways disease which are distinct from obstructive/PRISm spirometry. (page 3, line 107) - please add respiratory mortality in addition to all-cause and cardiovascular mortality (PMID 34905031 - this reference is also relevant for Discussion, p 9, line 346).

Reply 3: We thank the reviewer for these, again very helpful, comments.

Changes made in text:

- We have modified the sentence on p3, line 91, to indicate that chronic bronchitis and emphysema are historical subtypes, but not causes, of COPD.
- As we did indeed use spirometry data only, with obstruction (COPD) and PRISm as mutually exclusive categories, we have decided to delete the sentence on "mixed conditions" (with the Kinney reference) from the Introduction.
- We have added the words "respiratory mortality" to the sentence on page 3, line 107, with reference to PMID 34905031, and also added this reference to p 9, line 346, in the Discussion.

Comment 4. Methods / Results - mortality analyses. Please provide exact date of follow up for mortality data. In the Methods, follow up through 2022 is stated (p4, line 144), however in the Results state follow up was through July 2021 (p5, line 198). The authors should also include adjustment for well-established risk factors for all-cause mortality, including current smoking status, BMI, SES (education level can be used as a surrogate), alcohol use, and DM.

- Date of follow-up for mortality data; changes made in text:
  For the present analyses, the date of latest follow-up for mortality (occurrences of deaths) and data extraction was July 2021. Other dates originally mentioned referred to the (still) ongoing process follow-up, but have now been deleted to avoid any confusion.

Adjustments for established risk factors for all-cause mortality; Reply 4:
In fact, our models did already include detailed adjustments for smoking history (lifetime duration, average cigarettes /day, years since quitting) and BMI. Additional adjustments for self-reported diabetes mellitus (DM) hardly affected any of the relative risk estimates for all-cause mortality (<2% change in hazard ratio estimates), and therefore was not retained in the risk models presented. However, for all certainty, we have now replaced Table 6 with estimates from models that also include DM as adjustment (although HR are virtually unchanged)
Regarding SES: We are not very much in favor of additional adjustments for indicators of SES. SES is not itself a biological risk factor in itself, but at best an indirect indicator for still other, unknown risk factors for mortality. Furthermore, definitions of SES vary widely across countries and study populations, as well as its potential associations with mortality risks. Finally, and perhaps most importantly, we feel that accounting for SES in risk models may actually over-adjust and bias estimated associations of mortality with genuine biological risk determinants, such as COPD or PRISm.

- Changes made in text: We replaced the estimates ("Model 2") in Table 6, with estimates after the additional adjustment for DM.

Comment 5. Methods - (p4, lines 146-148) Clarify the cumulative smoking exposure (pack-years) to qualify for LCS (two qualifying criteria are listed: 15 cig/day x 25 years = 18.75 pack-years while 10 cig/day x 30 years = 15 pack-years); (p5, lines 182) "pulmonary edema" should be removed as this is NOT a pulmonary disease.

- Clarify the cumulative smoking exposure criteria; Reply 5: In LUSI, similar as is the well-known (Dutch-Belgian) NELSON trial (Koning et al NEJM 2020), eligibility for LC screening was not determined by pack-years, but through a slightly more complex algorithm based on combinations of minimal smoking duration and intensity. As precisely written in our text, the eligibility criteria were that participants should have cumulated either: (a) a minimum of least 25 years of smoking (lifetime) at an average intensity of at least 15 cigarettes a day, or (b) 30 years of smoking at an average intensity of at least 10 cigarettes a day. While these criteria correspond to a minimum of either 18.75 pack-years, or 15 pack-years, respectively, it would be wrong (and contradictory), to mention these two pack-year measures as our eligibility criteria.
Changes made in text: None

- (p5, lines 182) "pulmonary edema" should be removed:
Changes in text: Thank you for correcting us on this point; we have removed these words.

Comment 6. Methods - spirometry - (p5, lines 186-188): Was spirometry conducted according to ATS/ERS guidelines or were only 2 efforts obtained? This is critical to report to ensure that high-quality spirometry was performed; (p5, lines 190) - two different sets of reference equations are cited; which one was actually used? Also please clarify if all participants were of European ancestry or if other races/ethnicities were included. Please include this information in Table 1 as well.

Reply 6 / changes made in text:

- Spirometry guidelines; reply 6 / changes in text: Due to time limitations, spirometry measurements for FVC and FEV1 each were obtained only twice (2 efforts), and for both measures the "best" (highest values) were retained. This was already written in the text; hence, no changes made.

- (p5, lines 190), two different sets of reference equations are cited: Thank you for alerting us; this was an error. We used the equations of the Global Lung Initiative (reference Quanjer et al 2012). We deleted the other reference (Hankinson, NHANES-III), which additionally mentioned by mistake.

- Ancestry: Almost all LUSI participants (over 98%) were of white Caucasian origin. However, as ancestry information was not recorded, we prefer to only mention this as a general statement in the Methods description.
  Changes made in text: As we cannot precisely quantify the small number of persons with non-Caucasian origin, we prefer not to mention data about "race" or ancestry in **Table 1**. However, we did add a sentence to the Methods that "*Practically all participants are of "Caucasian" ethnic ancestry*."

Comment 7. Methods - statistical analyses - (p6, line 224) - t-tests were used, but how was non-normally distributed data handled?; (p6, lines 227-228) - Please clarify what "by replacing category indicator values with their class midpoints" was used for.

- t-tests, and non-normally distributed data; Reply: In fact, none of the variables for which t-tests were applied showed major deviations from normality, to the point that t-test results would have been seriously biased

Spirometry Categories	

- Replacing categorical indicator values; reply 7: Information on smoking duration (years), the time since quitting (years), and the average daily cigarettes was originally collected and coded in form of categorical variables: 5-year categories for lifetime smoking duration; time since quitting: 1; < 1 month, 2; 1-6 months, 3; 7 months – 1 year, 4; 1 – 2 years, 5; 3 – 5 years, 6; 6 – 10 years; average daily cigarettes categories: 3; 11-15, 4; 16-20, 5; 21-25, 6; 26-30, 7; 31-35, 8; 36-40, 9; 41-45, 10; 46-50, 11; 51-55, 12; 56-60, 13; >60 (as indicated in **Table 1** of our manuscript). We replaced category indicator values by their class midpoint values, so as to obtain quantitatively scored variables for analysis of risk association with smoking duration, average smoking intensity (cig/day), and time since quitting.

Changes made in text: To make this point clearer, we have added a sentence to statistical analyses section: "*Information on smoking duration, the time since quitting, and the average daily cigarettes was originally collected and coded in form of categorical variables (6 categories for lifetime smoking duration; 6 for time since quitting; 12 for average smoking intensity (cig/day)). To obtain quantitatively scored variables for smoking duration (years), the time since quitting (years), and average daily cigarettes category indicator values were replaced with their class midpoints*."

Comment 8. Results - The results should be consistently presented showing normal, PRISm, and COPD (GOLD1-4 inclusive) first before presenting the COPD subgroups (GOLD1, GOLD2, GOLD 2-4, GOLD 3-4, etc.). Similarly, for Tables 2, 3, 4, 5 - please either move the GOLD subgroupings to the supplement or at least consistently show GOLD1-4 before presenting the subgroups. In Table 4 - why was there no analysis of GOLD1-4 versus normal?

Reply 8 / Changes made in text: Following this advice, we have now retained a column only for COPD (Gold1-4) and PRISm in Tables 1, 2, 3 and 5. Results for COPD subgroups have been moved to the Supplemental Materials. Table 4 now also contains findings for GOLD1-4 vs normal.

Comment 9. Results - mortality analysis - FEV1/FVC ratio; would perform subgroup analyses stratified by lung function category. The effect of FEV1/FVC is likely not the same in COPD as in PRISm, yielding a null result in the analysis of the entire cohort.

Reply 9: We noted that we had actually wrongly cited our own findings (Table 6) in the text: In reality there is a clear inverse association between the FEV1/FVC ratio and mortality risk

Nonetheless, we also following the suggestion to perform further analyses on the association of the FEV1/FVC ratio, as well as of FEV1% predicted, with risk of all-cause mortality stratified by lung function category (i.e., for individuals with COPD or with PRISM); please see the tables below:

Spirometry Categories			
PRISm (N=311, 15.7%)			
FEV1 % predicted *	Model 1 Model 2	0.75 (0.53-1.05) 0.77 (0.54-1.10)	p=0.10 p=0.47
COPD (N=369, 18.6%)			
FEV1 % predicted *	Model 1 Model 2	0.84 (0.76-0.94) 0.84 (0.75-0.95)	p<0.01 p<0.01

* continuous variable (the odds ratios correspond to a unit of 10% increase)

Spirometry Categories			
PRISm (N=311, 15.7%)			
FEV1 / FVC **	Model 1 Model 2	1.23 (0.80-1.91) 1.36 (0.87-2.13)	p=0.77 p=0.74
COPD (N=369, 18.6%)			
FEV1 / FVC **	Model 1 Model 2	0.94 (0.79-1.13) 0.93 (0.77-1.13)	p=0.51 p=0.48

** continuous variable (the odds ratios correspond to a unit of 0.1 increase)

Model 1: adjusted for age and sex;
Model 2: adjusted for age, sex, lifetime smoking duration, average cigarettes/day, time since quitting (for ex-smokers)

Especially for the FEV1/FVC ratio we could not show clear-cut associations with mortality risk with the strata defined by lung function.Also, for both FEV1/FVC ratio and FEV1% predicted we found no statistically significant heterogeneity between the COPD and PRISm lung function categories (heterogeneity test not shown here).

Changes made in text: None.

Comment 10. Discussion - the unique contribution of this work is in demonstrating that PRISm is associated with increased (and in some instances, the highest) risks of lung cancer among LCS populations. This should be expanded upon. Important topics include the reported association between interstitial lung abnormalities (ILA) - which are different from clinically-diagnosed interstitial lung disease (ILD) - and an increased risk for lung cancer (Whittaker et al, PMID 31404527). Individuals with PRISm are enriched for ILA (Washko et al., PMID 21388308) - is information on ILA or ILD available in LUSI? Another important topic is the increased rates of transitions to other lung function categories / instability of PRISm. The authors should comment on whether PRISm on spirometry at a single time point, given the increased frequency of transitions in PRISm, impacts the value of LCS and life-years saved.

Reply 10: Again, we thank the reviewer for these very useful comments. We share the reviewer's opinion that our observation of increased lung cancer risk among individuals with PRISm deserve some more discussion (see our changes to text, below).

We also added a sentence to the Discussion to mention the single time-point ascertainment of PRISm, without further follow-up ascertainments, is a limitation of our study, and that this may have led to potentially imprecise (likely attenuated) relative risk estimates for lung cancer and for all-cause mortality.

Changes made in text: We have added two sentences text to the Discussion section to mention the association of (spirometry patterns indicating) PRISm with presence of ILA, as well as of ILA with lung increased cancer risk.

Minor Comments:

1. "Association of" should be "association with" throughout the manuscript.

Changes made in text: Thank you; we have modified this throughout the manuscript.

2. "Overall mortality" should be "all-cause mortality" throughout the manuscript.

Changes made in text: We made this change throughout the manuscript.

3. Results (p8, line 298) - the association between mortality and GOLD3-4 is NOT significant (OR 0.42-4.11); would revise statement.

Changes made in text: Thank you; we have revised our statement.

4. Table 1 - PRISm column, former smokers - should the percentage be 35.4% (instead of 25.4%)?

Changes made in text: We thank the reviewer for spotting this typo, and corrected this.

5. Table 4 – title states "CT-diagnosed emphysema" but these data are not presented in the table/or manuscript.

Changes made in text: We deleted the words "CT-diagnosed emphysema" (title of Table 2, not Table 4)

6. Tables 4 + 5: please change "known respiratory disease" to "self-reported physician diagnosed disease".

Changes made in text: Thank you – we have changed these terms