

Peer Review File

Article information: <https://dx.doi.org/10.21037/jtd-23-1061>

Reviewer A

Interesting paper. My main worry is that nowhere in the paper nor in the secondary methods references could I find a statement of the analytic sensitivity of the SEM method ie what is minimum fiber content detectable (content corresponding to one fiber counted) .

Eg with TEM and the counting protocol used analytic a sensitivity was 200000 f/gdl
(Leigh et al Cancer 68: 135-141 (1991).

If enough grid areas are counted and enough tissue sampled fibers can be found in almost everyone. Becklake Am J Resp Crit Care Med 1994;150:1488-1492

REPLY: we thank the reviewer for this important suggestion, we agree.

CHANGES IN THE TEXT: We provided the detection limit of our technique in M&M section.

With regards to the relative carcinogenicity of fiber types the Hodgson Darnton update should also be cited (Occ Env Med 2010; 67:432 ;Lentners etal Env Health Persp online 27 June 2011)

Other studies have shown associations between mesothelioma cell type and lung fiber content eg Leigh et al Cancer 68: 135-141 (1991). These should be cited.

REPLY: thank you for the suggestions, that contribute in improving the manuscript quality.

CHANGES IN THE TEXT: we added the suggested references and their relevant content in the discussion.

Reviewer B

The manuscript by Visona et al. entitled ‘Asbestos burden in lungs of non-occupationally exposed women from Broni (Pavia, Italy): a postmortem SEM-EDS study’ (JTD-23-1061-RV15-3445) is an interesting study investigating lung asbestos content of women living in the vicinity of an asbestos manufacturing facility. I have a number of comments for the authors to address.

The authors refer to the 1997 Helsinki document, which recommends that laboratories performing fiber burden analyses should provide their own background or control values in order to better interpret the results. The authors have not included such background data in this manuscript. Has this been performed? This is quite important in terms of implications for causation, since there is no scientific evidence that background exposures cause or contribute to the development of mesothelioma (Helsinki, 1997).

REPLY: we thank the reviewer for this important point. We agree about the importance

of this point.

CHANGES IN THE TEXT: We reported the background concentration that was observed in our laboratory investigating control subjects (individuals without any known exposure nor lung diseases) of similar age compared to cases.

Another important issue for consideration is the concept of detection limits. In cases where no asbestos fibers/bodies are detected, it is more appropriate to list these values as below the limits of detection (that is, less than the value represented by a single fiber detected). This is pertinent to the statements in the Discussion on pg. 10, lines 317-318. For example, the detection limits for asbestos body concentrations as determined by SEM are typically two orders of magnitude higher than the detection limits by optical microscopy (see Discussion, lines 322-324).

REPLY: thank you, we reported the detection limit in M&M, according to reviewer 1's suggestion.

CHANGES IN THE TEXT: We provided the detection limit of our technique in M&M section.

Concerning the Discussion on lines 343-344, it is important to recognize that what accumulates in the lungs drives what gets to the pleura. The lack of detection of chrysotile (in spite of exposure) in many cases likely reflects a less important role in mesothelioma causation, as indicated by two orders of magnitude greater potency for amphiboles in mesothelioma causation as compared to chrysotile (References 30, 31 in manuscript). Two more recent studies confirming these potency factors include Garabrant and Pastula (2018) and Korchevskiy et al. (2019).

REPLY: thank you, we totally agree that it was necessary to clarify this point.

CHANGES IN THE TEXT: we added these points, with references, in the discussion (lines 414-416)

In the discussion regarding half-lives of fibers in the lungs (Discussion, page 10, lines 305 and 307-308), 90 days is actually a half-life for chrysotile, so that it is not entirely cleared in that time period. Churg has reported amphibole half-lives of 10-20 years in humans. The authors explain the findings of non-commercial amphiboles on the basis of talc exposure (lines 353-356), but non-commercial amphiboles have also been reported to correlate with chrysotile exposure, since they are frequent contaminants of this latter fiber type (Roggli et al., 2002).

REPLY: thank you, we agree.

CHANGES IN THE TEXT: we added this explanation as well, specifying that not all the chrysotile ores are contaminated by amphiboles (430-432)

In the Discussion, pg. 9, lines 284-285, Pavlisko et al. (Ref. 4) analyzed lungs from 64 female subjects. Also in the Introduction, pg. 4, lines 115-116, Pavlisko paper also discussed household contact and environmental exposures.

REPLY; thank you, we agree.

CHANGES IN THE TEXT: we added such references where requested.

The title indicates that this is an SEM-EDS study. Morphological features alone do not permit

distinction among the various fiber types. Therefore, for Figures 1-5, please provide the corresponding energy dispersive spectrum for each back-scattered electron image of a fiber or asbestos body illustrated.

REPLY: we totally agree, this point will strengthen the paper.

CHANGES IN THE TEXT: we added the EDS spectra in each figure (except for figure 1, which represents an AB).

For Table 4, last entry, the mean width of short fibers can't be 0.0 (typo?).

REPLY: Indeed, it is a median, not a mean. This value is the median between the means of fiber widths in each case.

Also, in Table 6, for the correlation of fibers with latency, this should be an inverse correlation (higher the fiber concentration, the shorter the latency) as reported by Dragani et al. (2018). If this is what the authors found, they should indicate that it is an inverse correlation.

REPLY: this is, indeed, a direct relationship.

CHANGES IN THE TEXT: we explained more extensively the concept and compared our results with Dragani et al. in the manuscript (lines 486-488). Our data did not confirm that a higher asbestos burden can anticipate the onset of the disease.

Other minor comments are as follows:

- 1) Introduction, page 4, line 106, 'responsible of' should be 'responsible for'
- 2) Introduction, page 4, line 135, please define ARDs (asbestos-related diseases?) the first time used
- 3) Materials and Methods, page 7, line 201, 'was respected' should be 'was expected'
- 4) Materials and Methods, page 7, line 208, 'Texas, TX' is redundant ('TX' would be fine)
- 5) Materials and Methods, page 8, line 248, 'resulted' should be 'was found'
- 6) Lines 600-601, shouldn't this be in English?

This is a guideline in Italian, never translated, so we reported the original title.

7) Conclusions, line 403, 'regulated both asbestos and both regulated inorganic fibers and those shorter than 5 μ ' is awkward, and would better read as 'regulated asbestos and inorganic fibers as well as those shorter than 5 μ '.

REPLY: Thank you for the corrections, we amended the text as suggested.