Author's response to reviews

Title: Osteopontin, asbestos exposure and pleural plaques: a cross-sectional study

Authors:

Giuseppe Mastrangelo (giuseppe.mastrangelo@unipd.it)
Gianluca Marangi (gianluca.marangi@libero.it)
Maria N Ballarin (Nicoletta.Ballarin@ulss12.ve.it)
Silvia Michilin (Silvia.Michilin@ulss12.ve.it)
Aline SC Fabricio (aline.fabricio@ulss12.ve.it)
Flavio Valentini (flavio.valentini@ulss13mirano.ven.it)
John H Lange (jhlange1@hotmail.com)
Ugo Fedeli (ugo.fedeli@ulssasolo.ven.it)
Luca Cegolon (l.cegolon@gmail.com)
Massimo Gion (massimo.gion@ulss12.ve.it)

Version: 3 Date: 13 December 2010

Author's response to reviews: see over
Dear Editor,

I hereby re-submit the following manuscript entitled: “Osteopontin, asbestos exposure and pleural plaques: a cross-sectional study”, by Mastrangelo G et al.

The paper has been revised according to the manuscript of the two reviewers (please see below). I look forward to hearing from you soon.

Best Wishes.

Dr. Luca Cegolon, MD, FFPH  
*Corresponding Author*
ANSWERS TO REVIEWER 1

Comment 1
Ethical issue: even if medical surveillance for asbestos exposed workers is mandatory there are no guidelines for a systematic blood sample collection and assaying of any marker. Therefore if blood collection is not routine in the surveillance of asbestos exposed workers it is preferable to ask for an ethical approval in order to insure that the informed consent correctly informs the patient about the scope of the investigation. However this can be subject to variation according to national ethical regulations.

Answer 1
This research study was carried out within the context of a Regional Directorate Decree (DDR, Italian acronym) No. 48 of 20/12/2006 of the Veneto Region (please find the attachment: "Line for former exposed to Asbestos and CVM, and exposed to Asbestos"). This document is split into two components:
- PART A: Action plan (years 2005-2007);
- PART B: Financial plan (year 2006). The yellow marked part says: "Experimental health surveillance using blood sample tests" (in Italian).

Comment 2
There is no description of how the regression lines were drawn in the scatter diagrams in the methods section. Also the visual information supplied by the scatter diagram and the regression lines should be completed by presenting the equation of the regression lines. At the same time an univariate analysis should be done. Data should be presented along with the results of the multivariate analysis in table 4.

Answer 2
The regression lines were drawn in the scatter diagrams using the linear regression analysis. This is explained in the current version (see Methods, page 7, paragraph 3). The coefficients of regression are shown in table 2. Univariable and multivariate analysis are displayed in table 3 and 4.

Comment 3
The fact that authors generate more examined patients than sampled patients by including those lately addressed to the clinic can generate a considerable bias in the recruiting population and compromise the significance of the results.

Answer 3
Generalization can mean the statistical generalization from a sample to a larger basic population, or it can mean the scientific generalization from one particular study experience to the abstract-general or to the level of scientific theories. In epidemiological research, both types of generalization are made because the problem can be particularistic or abstract.

A particularistic problem is bound to time and space, and therefore studies of such problems are descriptive only. The study material is then a sample drawn from a larger basic population. Examples of such problems are the level of lead exposure in different types of companies in a geographical area or the prevalence of hearing loss in shipyards. Such studies are done from statistically representative samples or, say, lead-using industries in a city or all shipyards in a country. In such a setting, the generalization is one of sample-to-population. Provided the sample are correctly chosen, that is, statistically representative, their results can be generalized to the larger base-population.

The problem of generalization in scientific research are fundamentally different because in science the results of a study are being generalized beyond the data gathered from the particular study, and
from the particular base population, to the sphere of abstract knowledge or “scientific thrust”. Now it is irrelevant how statistically representative the sample was of, say, all factories or all workers in a country or in the whole worlds for the sake. Instead, the sample should represent a certain category of people, for example, male viscose rayon workers who have been exposed to carbon disulfide for at least 5 years. The purpose of scientific generalization is to determine if exposure to carbon disulfide in general causes or worsens CHD. For this problem it is totally irrelevant if the workers are representative of all Finnish men or if the plant is representative of all the plants of the world. The generalization concerns whether carbon disulfide is cardiotoxic. If so, the exposure (of a certain intensity and duration) to this chemical is dangerous for everyone. This is an example of the general, scientific conclusion which is the goal of scientific studies [from: Hernberg S. Introduction to Occupational Epidemiology, Lewis Publishers. 1992, pages 139-141]

In other words, the question may be either descriptive (which is the situation?) or analytical (which is the relation?); only in the first event a sample should be strictly representative of the large base population.

In our study, 904 workers previously exposed to asbestos – already examined at the Occupational Health Service (OHS) of the Primary Care Trust (PCT) 12 of Venice (Italy) – were stratified by cumulative exposure and pleural plaques (PP) and 30% of them were extracted from each stratum with a random sampling procedure. The final sample included 263 subjects, 54 with and 209 without PP. The percentage of response was as low as 63% in the group without PP. With a similar response percentage, the group with PP should have included 34 instead of 54 subjects. In order to avoid small numbers, some subjects lately addressed to OHS were included in the study, provided they had PP and a particular level of cumulative exposure. At the end of the study, in some subgroups the number of observed exceeded that originally sampled. This fact, in view of the above thoughts, cannot compromise the significance of the study, which concern the relation between the level of Osteopontin (OPN) and, on the other hand, the indices of asbestos exposure or the presence of asbestos related pleural/pulmonary disease.

**Comment 4**
The result section is far too short and should describe the result and not just state data is presented in table x, y or z. This information is supplied in the title of the table.

**Answer 4**
This section has been expanded and in the revised version results are fully described.

**Comment 5**
The authors should include in table 1 the numbers of the original populations from which the patients were sampled. As stated before only patients randomly selected should be included in the study.

**Answer 5**
The original table 1 has been removed and replaced by information supplied in the text of the current version. Please see:
- Study population, last lines of page 4 and first lines of page 5;
- Results, the first two lines, page 8;
- Discussion, second paragraph, page 11.

It was not possible to identify the 8 patients added later.

The rationale for this comes from Answer 3.

**Comment 6**
I would suggest that data be presented as scatter plots instead of tables since some of the parameters presented are clearly not normally distributed and hence the mean and standard deviation values are
meaningless (this is at least the case for peak exposure on very probably cumulative exposure data). These scatter plots should include values for mean and standard deviation (if variables normally distributed) of median and quartiles.

**Answer 6**

Several graphical methods and numerical tests have been used to check normality. However, for the sake of brevity, we have reported (figure 4) only the distributional diagnostic plots for OPN and 1/OPN, because they acted as dependent variables in the regression analysis. The Shapiro Wilk test, mean and standard deviation, median and quartile for all variables have been reported in table 1.

**Comment 7**

In the smoking parameters, age of starting smoking as well as the cumulative number of pack years should be included if the data is available.

**Answer 7**

We have that information, but current smokers were too few (15 without and 10 with PP). Therefore we have only used the classes of smoking.

**Comment 8**

There is no statistical comparison presented for any variable and any subgroup. The authors only state that some variables are higher in some subgroups but no clear definition of the test used or the exact p values is supplied.

**Answer 8**

In the revised version we have used:
- the test of Wilcoxon (table 1) to compare continuous variables (table 1);
- the chi-square to compare frequency of smoking classes (page 9, second paragraph);
- the Chow test to compare regression coefficients (table 2);
- several methods of regression diagnostics: checking normality of the dependent variable and residuals (figure 4); checking for independence of predictors (figure 3) and multicollinearity (tables 3-4);
- and the z test statistics provided by the linear regression analysis (tables 3-4).

**MINOR REVISIONS**

**Comment 9**

The legends for figures and tables should be more explicative.

**Answer 9**

Done.

**Comment 10**

The authors acknowledge the differences between various ELISA kits on the market and correctly state that results are not directly comparable. We have recently shown that for two kits available on the market these differences are the results of a proportional “error” effect. The information supplied and interpretability of the data would be improved if the authors would have the same plasma assayed also with another kit.

**Answer 10**

We thank the referee for this observation. As mentioned in the 'Subjects and Methods' section, in the present study we have a limited number of plasma samples simultaneously assayed also by
another kit. It was our intention to complete the comparison between methods in a larger cohort of samples. However, the limited financial resources available prevented the simultaneous examination of a large number of cases. Our preliminary data regarding agreement between methods are now described in 'Subjects and Methods'. Moreover, a paragraph was inserted in the Discussion.

**Comment 11**
Also the authors should discuss more in details the differences resulting from assaying plasma and serum since osteopontin is a molecule which is subjected to cleavage during the coagulation process.

**Answer 11**
We found the referee criticism on this point very useful, and added a whole paragraph in the Discussion on these aspects (“One could ask if there is any reason for assessing OPN levels only in plasma and not in serum....”)

Thank you for your comments.

Best Wishes.

Dr. Luca Cegolon, MD, FFPH  
*Corresponding Author*
ANSWERS TO REVIEWER 2

General comments

1
It should be made better clear whether the associations found are due to the confounding effect of age.
Answer 1
Done. Please see answer 7.

2
Some extra statistical work out is suggested.
Answer 2
In the revised version we have used:
• the test of Wilcoxon (table 1) to compare continuous variables (table1);
• the chi-square to compare frequency of smoking classes (page 9, second paragraph));
• the Chow test to compare regression coefficients (table 2);
• several methods of regression diagnostics: checking normality of the dependent variable (figure 4); checking for independence of predictors (figure 3) and multicollinearity (tables 3-4);
• and z test statistics provided by the linear regression analysis (tables 3-4).
Please see also answer 8 and 11.

3
Limitations of the study should be better discussed.
Answer 3
Done. Please see answer 16.

Specific comments

4
Abstract: The results section gives some contradictory information. Reformulate "The explanatory variables with significant influence on Ostepontin were length of exposure (positive correlation) and time elapsed since last exposure (positive correlation)." to stand "In a multivariable regression model with backward elimination, the explanatory variables with significant independent influence on Ostepontin were length of exposure (positive correlation) and time elapsed since last exposure (positive correlation)."
Answer 4
Done

5
Background: The authors state: "The present investigation aims to assess the relationship between plasma OPN levels and asbestos exposure or presence of asbestos-related diseases..." Out of asbestos-related diseases, only pleural plaques have been studied.
Answer 5
The sentence has been rephrased since there were no cases of asbestosis in our series.
Materials and methods: How was asbestos (peak and cumulative) exposure assessed?

Answer 6
This is explained in the revised version (please see section “Assessment of historical asbestos exposure” beginning on page 6).

Materials and methods: Were there any collinearity problems between the independent variables?

Answer 7
The issue of independence of predictors and checking for multicollinearity are explained in the current version (please see Statistical analysis, page 9). The corresponding results are reported in figure 3, and in tables 3-4; and are presented in Results (last paragraph of page 10, continuing on the next page) and Discussion (page 13).

Materials and methods: Was the frequency distribution of OPN close to normal?

Answer 8
The normality of the dependent variables (OPN and 1/OPN) has been checked with graphical methods (figure 4) and numerical tests (Shapiro Wilk test).

Materials and methods: The authors have studied the relation between pleural plaques (as an indication of asbestos exposure) and OPN. Similarly interesting could be the relation between lung fibrosis and OPN. Do you have data on this?

Answer 9
In our series there were no cases of asbestosis.

Materials and methods: How was current/former smoker defined? I.e. if the person smoked the latest cigarette one week ago, which category she/he belongs to? It is common to use pack-years as a cumulative measure of smoking.

Answer 10
The smoking categories are now defined (please see Statistical analysis, second paragraph of page 8). We have the relevant information to calculate pack-years, but current smokers were too few (15 without and 10 with PP). Therefore we have only used the classes of smoking.

Results: I would like to see a table indicating bivariate regression coefficients between all independent variables and OPN. What happens if age is thereafter forced to such regression models as a single covariant? Will the significant relations between OPN and asbestos exposure disappear? Consider an extra table focusing on this.

Answer 11
This has been done (please see table 3 and 4; Statistical analysis, first paragraph of page 9; Results, page 10; and Discussion, last two paragraphs of page 13 continuing on page 14).

Results: Table 3 may be somewhat irrelevant. Consider removal.
Answer 12
The old table 3 has been removed.

13
Results: Fig. 2: Do not fit a line in the plot describing OPN and smoking class (the latter is not a continuous variable). The same probably holds true between OPN and peak exposure (only 3 classed described).
Answer 13
We have tried but the new figures (without lines of tendency) were always different from the others in some format detail. So they have not been changed.

14
Discussion: Is the 1st paragraph really necessary? Consider removal or shorten it.
Answer 14
This paragraph has been shortened and moved below in Discussion.

15
Discussion is often started a short referral to main results.
Answer 15
In the present version, Discussion begins with the aims rather than the main results because there are main results.

16
Discuss the problems of your study not concentrating solely on OPN.
Answer 16
In the new version we have included four limitations of the study (Discussion, pages 12-13).

Thank you for your comments.

Best Wishes.

Dr. Luca Cegolon, MD, FFPH
Corresponding Author