Author's response to reviews

Title: Fungal exposure in homes of patients with sarcoidosis

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Version: 2 Date: 27 September 2010

Author's response to reviews: see over
We thank the reviewers for excellent comments. We have made an extensive revision of the ms and corrected all subject data.

Reviewer 1.
- Major Compulsory Revisions

1/ methods page 6: They are several concerns about the description of the patients:
- you should give the stage of sarcoidosis for each patient, before a treatment was initiated.
- you should give the used criteria to treat the patients with corticosteroids.
- on page 6 line 6, you said that stage 2 sarcoidosis were treated; as the stage 2 is not an indication of treatment, could you give the reason of this treatment

A. We have now inserted information on diagnostic criteria as well as the characteristics of the group chosen for home measurements.

• it seems that among the 104 sarcoidosis, only 5 patients did not receive oral steroids. That means that more than 90% of your patients were treated with systemic steroids which is too high in regard with the classical recommended indication of treatment.

A. One explanation is that the group studied does not represent a random sample of sarcoidosis in the population but is from the group of patients attending a particular clinic in the city center. Anyway this does not influence the conclusions

• did some patients present extra-thoracic sarcoidosis (heart? uveitis? other?).

A. Information on the number with extrapulmonary manifestations has been added. A detailed list would not add any valuable information.

• the age of the populations is surprising (> 45 years) because the prevalence of sarcoidosis is maximal between 20 and 40 years.

A. Again it might be a selection of patients to the particular clinic.

2/ Methods page 7: what is the repeatability of the NAHA measurement, of air
collected at different time in a given house?

A. Data have been added

3/ you should give a description of the presence of moisture and markers of humidity in the home of the sarcoidosis compared to the controls.

A. An ocular inspection was not done in a systematic fashion as we relied on NAHA values for the classification. Also recent studies demonstrate a poor correlation between ocular description and measurements of fungal cell agents (Reponen et al Sci Tot Env 2010;doi:10-1016.

- Minor Essential Revisions
  - abstract line 8 : there were less than 290 healthy subjects!
    A. Typo corrected
  
  - abstract – results : « compared to control, subjects undergoing treatment… » instead of « Subjects undergoing treatment… »
  
  - abstract – results line 3 : define what is the treshold « 13 U/m3 »?

  - abstract – results line 5 : « 13 U/m3 » instead of « 13 »

  - abstract – conclusion : « The higher levels of NAHA.. » instead of « The higher levels of enzyme.. »
    A. Corrected


A. There are already two references to fire-fighters and as this is not a review we don’t think it is necessary to add another reference here.

  - Methods para 1 line 3 : « patients » instead of « patents »
    A. corrected

  - Methods para 1 line 4 : « a transbronchial biopsy.. » is not enough. Generaly several repeated biopsies are required to obtain significant results.
A. The routine at our clinic is to make one biopsy. Occasionally two or three are required. The text has been adjusted. See also comment above.

**Methods:**

- Table 1: you should add the normal values of each parameter. SE or SD or CI

A. We don't think normal values would give any information as we are making comparisons between the three groups studied

- Page 7: Why did you express the NAHA activity as « EU » in the methods and as « U » in the results?

A. Corrected

- Table 3 compared to table 1 and 2: the number of subjects in each group is not consistent! (for example 28 versus 30 controls)

A. Corrected

**Reviewer 2.**

Reviewer: Hajime Goto

Reviewer's report:

This paper deals with the fungal biomass levels in homes of patients with sarcoidosis. The authors found that the levels in homes of patients with active sarcoidosis was higher than inactive sarcoidosis or controls. As the pathogenesis of sarcoidisis has not been made clear, the findings shown here are interesting. Several comments of the level of “minor essential revisions” are as follows:

1. Patients were classified into three groups; sarcoidosis less than a year, sarcoidosis with recurrence and sarcoidosis without recurrence. However, among these three groups, there were no difference in the markers of the activity of the disease; CD/CD8 ratio or ACE level, etc (Table 1). The authors should show the rationale to classify the patients into three groups, especially the criteria to differentiate the patients into recurrence group and into no-recurrence group.

A. Information on classification has been added

2. In no-recurrence group, NAHA levels were low. On the other hand, it is
suggested that mould levels in a certain room were vary relatively little (reference 11). Presumably, there were no differences of the environmental conditions in the homes of no-recurrence group patients. What is the reason of the decline of NAHA levels in homes of no-recurrence group?

A. Very relevant comment. The reasons were probably cleaning and sometimes moving to another home. No systematic follow-up was made but is planned for the continued studies

3. The authors classified the patient with the second criteria of NAHA 13 U/m3. The rationale to set NAHA 13 U/m3 as the cut off level should be shown.

A. The value refers to the second quartile. The information is already in the text page 9.

Reviewer 3.

The authors’ goal was to examine whether fungal exposure in homes was associated with clinically established sarcoidosis in a case-control study in Slovenia. Although the article fits to the scope of the Journal, the manuscript suffers from methodological flaws and part of the sections are not adequately delineated. Furthermore, the manuscript would benefit from improved sentence structures and use of proper grammar. The manuscript presents information that would be of interest, but several issues limit the impact of the paper and reduce enthusiasm.

A. We have revised the manuscript extensively

Major comments
1) The introduction is short and does not review the background adequately. Sarcoidosis is a disease of unknown etiology, but there is increasing evidence that microbial antigens may play a role in the pathogenesis. Although fungal exposure may contribute to the development of sarcoidosis, other agents (e.g., Propionibacterium, Mycobacterium) have also been considered important in etiology (Oswald_Richter and Drake, Semin Respir Crit Care Med 2010:31:375-9). While several epidemiological studies have reported adverse health effects with fungal exposures in indoor environments, the exact
inflammatory and immunological processes behind the observed associations between the exposures and health outcomes are still largely unknown.

**A.** We agree that other microbiological agents have been discussed but we did not want to extend on a review of the previous (mostly negative) studies and thus have only one general reference (1) acknowledging the broad scope of microorganisms that have been discussed.

2) The authors give very little details on the sampling procedures. Readers may not be familiar with N-acetylhexosaminidase assessment method, which was developed to provide more practical method to quantify fungal biomass (ergosterol). For example, methods allowing quantification of mold on building materials are important when evaluating mold damage in buildings and the quality of the remediation efficacy. While concern about indoor mold exposures has increased over the past decades, the complexity of the fungal exposure assessment may not be clear to all readers.

**A.** We have added details about the sampling and analysis procedure as well as the exposure to spores and fractions of fungal cell walls.

3) It is well known that molds are commonly found in both outdoor and indoor air. Interpretation of possible indoor fungal exposure can be addressed using (1) indoor/outdoor total concentration ratios, (2) comparisons of the species compositions detected indoors and outdoors, and/or (3) the presence of indicator species that are associated with excess moisture problems (e.g. Aspergillus and Penicillium spp.). Did the authors assess fungal biomass concentrations outdoors? It is likely that the results from a sampling conducted at a single point in time may not represent exposure throughout the entire year because fungal exposure is prone to temporal, particularly seasonal, and spatial variations (LeBouf et al., J Air Waste Manag Assoc 2008;58:684-92).

**A.** No sampling was not made outdoors. Doors and windows were closed prior to the sampling, ascertaining real indoor exposure conditions. Previous studies have demonstrated a rather low variation indoors over time (reference 11).

4) Indoor exposures are of great importance in relation to many health outcomes because most people spend a large amount of their time indoors, especially at
home. The authors do not consider potential confounding by other indoor air pollutants (e.g., emissions from gas/wood stoves, environmental tobacco smoke, pets, dust mites, or other indoor allergens) in their manuscript. Indeed, there is emerging evidence that cigarette smoking is a strong risk factor in the otherwise unknown etiology of chronic diseases. Can the authors think any other factors that could enhance inflammation in the cases? Do the total levels of total IgE suggest presence or absence of allergic inflammation? Furthermore, recent findings suggest that nutritional status may also influence sarcoidosis status (Boots et al., Respir Med 2009; 103:364-72).

A. These comments are all adequate and it is possible that a simultaneous exposure to other agents may aggravate the reaction to fungi. We have added information on other factors in the text. Whatever the complete exposure situation, we find it rather remarkable that a single indicator of mould exposure gives such a good correlation with active forms of the disease.

5) Controls should be selected from the same population that gives the rise to the cases (Rothman and Greenland, Modern Epidemiology, 1998). Were all cases non-smokers like the controls (p.6)? In the abstract, the number of controls is 290 (p. 2) – however, in the tables the number of controls range from 28 to 30 (Table 1, 2, 3). How many controls were chosen for the study?

A. The controls were selected from the same base population (inhabitants in Ljubljana). There were 28 controls (table 1). Figures in the abstract and table 3 have been corrected.

6) The presented results are unadjusted – no potential confounders were considered in the analysis. Any comments?

A. The aim was to study a specific exposure and we found significant relationships – we did not address the question of confounders. We cannot think of any confounders that are related to fungal cell wall exposure and sarcoidosis.

7) Socioeconomic status (SES) is often closely associated with health-related outcomes, and type of housing can influence indoor exposures. Did the authors collect any information on these factors?
A. No this was a straight exposure - response relationship and we don’t see how social class could have influenced the conclusions. Of course it is likely that there were social class differences between the groups but that is of less interest in view of the hypothesis regarding a relationship to a biologically active agent.

8) An expanded discussion is warranted; the discussion in its current form is too short and it is not very well structured. The authors should carefully discuss differences and/or similarities of their findings with respect to previous findings. Furthermore, the authors should emphasize the novelty of their study design.
A. The discussion has been reworked. Please note that this is the first study demonstrating a significant relation between an airborne possible causative agent for sarcoidosis and the disease. There is thus not so much to review from previous studies.

9) The manuscript would benefit from improved sentence structures and use of proper grammar.
A. Corrections have been made

Minor comments
1) The paragraph describing the study subjects should disclose information about consent procedures.
A. OK, included

2) Population characteristics are generally presented in the Results section.
A. Varies, we look upon this information as background data and not real results

3) If the results were highly skewed, why the authors present mean concentrations? For skewed distributions, geometric mean is usually better measure of central tendency.
A. This would not change the conclusions

4) Proofread the manuscript carefully, correct typographical errors (e.g., add “comparable” to the first sentence on page 7 – “The groups were relatively comparable, although…”)
A. OK done