Author's response to reviews

Title: Neutrophilic airways inflammation in lung cancer: the role of exhaled LTB-4 and IL-8

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Title: Neutrophilic airways inflammation in lung cancer: the role of exhaled LTB-4 and IL-8

Dear Professor Paolo Bruzzi,

thank-you for the review of our paper: “Neutrophilic airways inflammation in lung cancer: the role of exhaled LTB-4 and IL-8”.

We are very grateful for all your helpful comments.

We have revised our manuscript according to the reviewers’ comments and highlighted the changes in bold.

Our replies to the specific comments of the reviewers are as follows:

Review 1

Reviewer's report:

This is a study from a well-known research group that addresses the role of exhaled LTB-4 and IL-8 from patients with NSCLC. The authors demonstrate that higher levels of LTB-4 and IL-8 are present in breath condensate and in the blood from NSCLC patients cancer and that LTB4 and IL-8 concentrations increase as the cancer stages progress. Moreover, exhaled IL-8 and LTB-4 correlate with the percentage of neutrophils in the induced sputum. The topic of this paper is of interest and some novel findings are described in this manuscript.

There are some points of the manuscript to revise.

C1. Introduction.

a. The Introduction may be condensed. The authors have to simplify and clarify
The aim of the present study was to give further support to the neutrophilic inflammatory signalling in lung cancer investigation, two soluble neutrophilic proteins, the LTB-4 and the IL-8 in the EBC of NSCLC patients and controls with a view to giving further insight into the inflammatory theory of lung cancer pathogenesis.

b. Please, add the references to the sentence “often a good number of studies are available in literature proposing the use of this sample for the non-invasive diagnosis and monitoring of lung cancer”.

R1. a: Thank-you for your useful comment. We have condensed the introduction and simplified the last sentence as suggested.

b: We have put references to the sentence indicated and furthermore we have added new relevant references.


a. The authors reported in the statistic section that “Data were normally distributed and therefore non–parametric tests were chosen for analysis”. I think that the authors have forgotten the word not normally distributed because non parametric tests are preferred for not normally distributed data.

b. In this study, smokers (without lung cancer) have been omitted. Therefore, while the presented data are interesting, it is not fully elucidated whether IL-8 or LTB4 levels are increased by lung cancer disease or represent a smoking effect only. In this regard it has been demonstrated that immunoreactive IL-8 and LTB4 significantly increased in the supernatant fluids from airway epithelial cells in response to smoke extracts (Masubuchi T, Am J Pathol 1998- Pickett G, Toxicol Sci. 2010). Furthermore the authors of the present study reported that increased levels of both exhaled LTB-4 and IL-8 were observed in heavy smokers (group 2) with respect to group 1 mild smokers. The evaluation of LTB4 and of IL-8 also in exhaled breath condensate from smokers without lung cancer or the presentation of LTB4 and of IL-8 data in non-smokers, current or ex-smokers lung cancer patients as separate groups might allow to fully elucidate the effect of lung cancer in the levels of these mediators and to support the utility of assessing these mediators in the diagnosis and monitoring of NSCLC.

R2: a. Thank-you for your comment. We are sorry. We didn’t in fact mean “not” normally distributed. We have corrected the text accordingly.

b. Thank-you for your comment. We agree that it is important to elucidate whether IL-8 or LTB4 levels are increased by lung cancer disease or represent a smoking effect only. In this regard, we have added a new group of healthy smoker subjects as suggested. Although healthy smokers showed higher levels of both markers compared to non-smokers, the concentrations of exhaled IL-8 and LTB4 were significantly higher in NSCLC patients, suggesting that their increase is not only due to cigarette smoke but also to the effect of lung cancer. We have added these new data in the result section and as figure (figure 3) and discussed them in the text.

C3. Discussion.

a. In the discussion the authors state that “……... confirmed the increase of LTB-4
and IL-8 systematically and in airways”. This reviewer think that it is better: “confirmed the increase of LTB-4 and IL-8 in the systemic compartment and in the airways” rather than “systematically and in airways”.

b. This reviewer thinks that it is better mollify the concept that the increase in LTB4 and in IL-8 represents a proof that neutrophils or inflammatory cells are the main source of these mediators. Airway epithelial cells may actively contribute to the release of both LTB4 and IL-8 and the correlations between these mediators and the number of neutrophils may be the results of the chemoactrant or pro-survival (Petrin D, Cell Signal 2006-Fudala R, Am J Physiol Lung Cell Mol Physiol. 2007) properties of these mediators.

R3: a. Thank-you for your comment. We have modified the sentence as suggested.

b. Thank-you for your comment. We agree that the concept that 'the increase in LTB4 and in IL-8 represents proof that neutrophils or inflammatory cells are the main source of these mediators' is highly speculative and have therefore modified the sentence as suggested, adding the Petrin and Fudala references.

Minor

C1: Present references in a separate section (the Heading “References” is located in the last page of the Discussion section).

R1: Thank-you for your comment. We have re-located the heading “references on a separate page.

C2: Add statistic in the legend of figure 1.

R2: We have added statistic to the legend of figure 1, as suggested.

C3: Add statistic in the figure 2 as well as in the legend of figure 2.

R3: We have added statistic to the legend of figure 2, as suggested.

C4: Revise further the paper for the English (discussion page 14: “previous” instead of “preceding” results).

R4: A native Englishman has revised the manuscript

Review 2

C1: The manuscript is interesting but I think it is still incomplete. There are many mistakes in writing; for example, there is no figure 3 in the manuscript although they mentioned it in the text. Also, in Table 1, the number of adenocarcinoma (histotype) is wrong it should be 28 not 18. Additionally, there are many spelling/grammer errors; for example, in page 17 (references part), reference 1 and reference 2.

R1: Thank-you for your comment. I’m very sorry, but there were typographic mistakes that we have now corrected in the text, accordingly.

Figure 3 refers to the effect of smoking on exhaled LTB4 and IL-8 concentrations (lacking in the previous version of the manuscript). We have now added figure 3 with new data on healthy smoker subjects.
In the paper entitled "Neutrophilic airways inflammation in lung cancer: the role of exhaled LTB-4 and IL-8", Carpagnano et al. detected the high concentration of LTB-4 and IL-8 in breath condensate and blood of patients with NSCLC. Authors expected that these exhaled protein could be a biomarker of NSCLC.

C1: In the results section (page 10-11), authors described the correlation between smoking habits and concentration of LTB-4 or IL-8 in the EBC (Figure 3). However, the manuscript lacks Figure 3 and the legend.

R1: Thank-you for your comment. We are very sorry that figure 3 was lacking in the previous version of the manuscript. We have included it, adding new data on healthy smoker subjects. We have also added the relative figure legend, as suggested.

C2: Authors showed the total number of patients as 50 in table 1. However, it was also shown that the number of male and female were 26 and 14, respectively, and 22 patients with squamous cell carcinoma and 18 patients with adenocarcinoma.

R2: Thank-you for your comment. I’m very sorry but there were typographic mistakes that we have now corrected in the text, accordingly. Male and female numbers were 36 and 14 respectively, whereas patients with squamous cell carcinoma numbered 22, and those with adenocarcinoma numbered 28.

Hoping that the revised version of our manuscript meets the selection criteria of your Journal,

Sincerely,

Giovanna Elisiana Carpagnano.