Author’s response to reviews

Title: Stimulated monocyte IL-6 secretion predicts survival of patients with head and neck squamous cell carcinoma

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Version: 4 Date: 5 October 2007

Author’s response to reviews:

Dear Editor!

Please find enclosed the revised manuscript entitled "Stimulated monocyte IL-6 secretion predicts survival of patients with head and neck squamous cell carcinoma". Revisions have been made as suggested by the reviewers and their comments are responded to in this letter.

In general:

a) The manuscript has been corrected by a native English language speaker.

b) The use of adequate statistics methods have been ensured by a professional statistician as stated in the acknowledgements.

c) The first version of the manuscript contained only survival results as to IL-6 secretion. This because only the IL-6 results yield significant prediction measured with original data, with binomially transformed data as well as with and without adjustment for TNM. After having read the response by the reviewers we decided to include the MCP-1 survival data in the manuscript as well because the MCP-1 data binomially transformed yield to some extent a survival prediction. By doing this, it becomes more understandable why the original tables 3 and 4 were included, both providing important information, with both dichotomised and not dichotomised values with adjustment for age, gender as well as TNM stage of patients. We have, however, stated in the Discussion (first paragraph) that the MCP-1 survival prediction only depicts a hypothesis that should be further studied.

Specific corrections in accordance with the suggestions from reviewer M. Rita Young:
a) The data showing the levels of IL-6 produced by patients that were alive or
death after 5 years are now presented in a figure (Fig. 3). The data presented in
the text are in accordance with the conclusion, which state that a high LPS
induced monocyte IL-6 secretion correlates to worse prognosis.

b) Range of cytokine production as well as median values are now given in the
text (Material and methods section).

c) The baseline (or control) values of cytokine production in monocyte cultures
not stimulated with LPS are now shown in all figures as suggested. IL-6 levels in
serum of HNSCC patients have been measured with the similar ELISA kits as
used in this study. We found that most serums (both from patients and controls)
had only low levels of IL-6. Therefore it does not seem likely that the amount of
cytokines included in serum add notably to the measured levels of cytokines in
LPS stimulated monocyte cultures. It is also interesting to note that the IL-6 SFM
condition provided the best survival prediction.

However, the influence of autologous serum on monocyte function is interesting.
It has previously been shown that the baseline production of MCP-1 from
monocytes may be decreased when monocytes are stimulated with LPS in
cultures supplied with autologous serum. In this study we observed that the
decrease in MCP-1 secretion from LPS stimulated monocytes compared to
baseline is even more pronounced when serum free conditions are applied (as
can be seen in figure 1b and 2b). As the main focus of this study was to reveal
whether monocyte function as measured by IL-6 and MCP-1 secretion correlated
with prognosis of HNSCC patients, we have not studied the effect of different
serum sources further. It is, however, an interesting question and we will certainly
look into this topic more thoroughly in future studies.

Specific corrections in accordance with the suggestions from reviewer Zhong
Chen:

a) The number of patients and controls are now matching in the text and in table
1. The correct number of HNSCC patients (65) and controls (18) are now clearly
stated. The diagnoses of the control patients are now described in the text. Table
1 and 2 are difficult to combine as suggested and we therefore have decided to
remove the original table 2 and place this information in the Results section only.

b) The monocytes are stimulated without any delay after isolation and this
information is now explicitly given in methods section. The terms \( \text{directly}, \)
\( \text{continuously}, \) and \( \text{dichotomized} \) are strictly related to how the results of
cytokine analysis are adapted to the statistical analysis. In order to avoid mix-up
of terms we have changed the text both in table headings, figure legends as well
as in the result section of the manuscript.

c) The baseline productions of monocyte IL-6 as well as MCP-1 prior to
stimulation are now shown in the figures 1, 2 and in the new figure 3 (see also \( \text{c} \)
above). The statistical tests used are shown every time a p-value is given as
suggested.

d) The values referred to as ‘not shown’ in the second paragraph in the results (original manuscript) were the baseline production of monocyte MCP-1 prior to stimulation and it is correct as assumed by the reviewer that the negative values come from reduced stimulated levels compared to the baseline levels. This is now more clearly shown in the figure 1 and 3 and stated also in the text as suggested by both reviewers.

e) There is a difference in LPS stimulated monocyte cytokine production when autologous and serum free medium culture conditions are compared. However, the basal levels of IL-6 and MCP-1 in monocyte cultures did not show any difference when cancer and control patients were compared. Neither did baseline cytokine levels have any predictive value as to survival. Furthermore, the levels of IL-6 in the serum are generally low (see ‘c’ above). Finally, the cytokine levels used in tables 2-3 and in figure 4 are all from monocyte cultures with serum free conditions. Still, we find this remark justified and have added comments about serum factors in HNSCC patients in the discussion section of the manuscript as suggested by the reviewer.

f) The original tables 3-4 (now 2-3) are better described both in legends and better cited in the results section of the manuscript. The original table 5 is removed and the information only given in the results section. We have added a new table (4) showing to what extent monocyte MCP-1 secretion predicts survival.

g) More recent publications of the regulatory mechanisms of IL-6 and NF-kB in HNSCC are cited and discussed as suggested.

After this revision we hope that the manuscript is acceptable for publication.

Yours sincerely,

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