Reviewer's report

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

Version: 2 Date: 18 July 2007

Reviewer: Zhong Chen

Reviewer's report:

General

--------------------------------------------------------------------------------

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

The manuscript entitled “Stimulated monocyte IL-6 secretion predicts survival of patients with head and neck squamous cell carcinoma” by JH Heimdal et al, studied monocytes in vitro production of IL-6 and MCP-1 in response to endotoxin, and revealed an association of the cytokines production with the prevalence, stage and prognosis of head and neck squamous cell carcinoma (HNSCC). Following sixty-five patients for five years, they found that monocytes from HNSCC patients produced higher IL-6 and MCP-1 in vitro in response to stimuli when compared to controls. In patients, increased in vitro monocyte-derived IL-6 production was associated with decreased survival rate, which is retained when adjusted for age, gender, disease stage, and ESR and albumin levels. They concluded that increased monocyte responsiveness to endotoxin as measured by increased IL-6 secretion is an independent negative prognostic factor for HNSCC patients.

The study is significant for head and neck cancer field, and contributes to the notion that the inflammatory cytokines could be used as the predictive biomarkers to assess patient prognosis. Previously, inflammatory cytokines, especially IL-6, has been extensively studied and reported as prognosis biomarkers in serum for HNSCC (Ref 1-3). The responsiveness of monocytes in cytokine production, and its prognosis value for five year survival of HNSCC patients have not been well studied. The conclusion drawn from this study could be important for future determination of monocyte functions and inflammatory cytokines in the prognosis of HNSCC patients. However, the manuscript is poorly written, the presentation of the methods and results lacks clarity, and at present form can not be considered as acceptable. The reviewer suggests a major revision of the manuscript before the acceptance for publication.

The specific comments are:

1. In “Methods”, it is not clear whether the patients with benign head and neck diseases (n=18) were served as control subjects. The authors need to briefly describe what kind of benign conditions in the control cohort. Tables 1 and 2 can
be combined into one table summarizing clinical characteristics. In the “Methods”, the number of HNSCC patients was 65, and the number of patients with benign diseases was 18. However, in Table 2, the number of HNSCC patients became 61, and the number of control patients was 19. Please reconfirm how many patients were used for this study and explain the discrepancy.

2. In “Methods”, it is not clear that after monocytes isolation, how long the monocytes were cultured before the stimulation, and how long the stimulation was carried out before harvesting the conditioned medium. Please explain more clearly the experimental conditions, and define the experimental conditions as described in the figures and tables, such as “directly”, “continuously” or “dichotomized” measured cytokines. The reviewer has a hard time to understand and judge the data of Tables 3-5 with these undefined terms.

3. In “Results”, first paragraph, please state if the authors observed a difference in the baseline production of monocytes IL-6 prior to stimulation. It is nice to see baseline levels of cytokine production in figures (see more discussion in comment #5). When comparing stimulated IL-6 levels in cultures and found significant levels p<0.05, the authors need to explain the statistical test used. Please specify statistical tests performed for all places where p values were used.

4. In the second paragraph, the authors stated that “The level of MCP-1…. (not shown)”. However, the authors presented MCP-1 data in Fig 1B. Are these two different sets of data? What are the differences? Also, cytokine production is rarely presented as negative values. The reviewer assumes that the negative values come from the reduced stimulated levels than the baseline levels. The reviewer suggests present both baseline and stimulated levels for both IL-6 and MCP-1, to clarify data presentation.

5. The authors noticed that they obtained different conclusions of the cytokine production in monocytes cultured in autologous serum versus serum free conditions (Figure 1). The authors should show the basal levels of IL-6 and MCP-1 cytokines without endotoxin treatment. Are the basal levels different between cancer patients and controls? There are several previous reports indicate that the serum from patients contains significantly higher inflammatory cytokines (Ref 1-3), which could confound the endotoxin stimulation results. The authors should discuss these related mechanisms.

6. Tables 3-5 should be better explained with legends and in the results. Why you need three tables? What different conclusions or information would you get from the different tables? According to the table titles, the difference of Table 3 and 4 is “continuously measured cytokines” versus “dichotomized cytokines”. However, the statistical methods presented in the two tables are different, one is regression co-efficient, plus SE and p value (Table 3), and another is relative risk, 95% CI (RR) and p value (Table 4). The authors did not give the rationale why they used different data or presentation, what difference in statistics for the data presented? In addition, the p values for IL-6 in these tables did not always reach statistical significance, which is not consistent with the conclusion. The
reviewer suggests consult with a statistician to revise the tables for a better presentation of these data.

7. More recent publications of the regulatory mechanisms of IL-6 and NF-kB in HNSCC could be cited and discussed (ref 4 and 5).

References:


**What next?:** Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interests.