Reviewer's report

**Title:** Transcript levels of different cytokines and chemokines correlate with clinical and endoscopic activity in ulcerative colitis

**Version:** 2  **Date:** 11 September 2008

**Reviewer:** Stig Larsen

**Reviewer’s report:**

General Comment:
The topic is important, the manuscript is well written and the aim of the paper is clearly described.

However, the statistical method use is not suitable for answering the aim. Simple Pearson Linear Correlation Analysis can not answer the question raised in the aim, but only estimate the correlations between pairs of variables.

In order to fulfil the aim, Multiple Regression Analysis or similar statistical model with CAI and EAI as dependant variables and the remaining variables as independent have to be performed. The set of independent variables such as CXCL8, CXCL10, CXCL2 and calgranulin # are obviously internally correlated. It might be they are even collinear. Most probably one or more of these will be dominant and overrule the impact of the other. Correlation Analysis will not be able to discover that unless Partial Correlation or even better Principal Component Analysis is added. However, in order to establish a group of biomarkers which can reflect mucosal inflammation in ulcerative colitis, Multiple Regression Analysis is recommended.

The material includes 49 biopsies from 27 patients. Thus, data from one patient might be included several times in the analysis. It is stated in the manuscript that the time interval between the measurements in these cases are at least 3 months. However, there are correlations within patients. To which extend this will influence the outcome of the analysis is however not obvious. My suggestion is to perform a Nested Multiple Regression Analysis with the patient as the nesting factor. If so, the influence of the within-patient correlation will be taken care of.

Conclusion: In order to answer the aim of the paper, the material has to be reanalysed by using a statistical model like Multiple Regression Analysis with the patient as the nesting factor.

I do not recommend the manuscript published before this major revision is performed.

Discretionary Revisions:

Abstract:
1) Number of biopsies and participating patients are lacking.
2) The estimated correlation coefficients have to be given, not only the p-values.
3) It is stated in the conclusion that the data given shows that real-time PCR quantification is a valid method. It is not given any results in this paper neither for specificity nor sensitivity of the method. How can they then claim validity?

Statistical Analysis:
1) ANOVA is arbitration for Analysis of Variance. To the best of my knowledge, such statistical model is not included in the analysis of the data presented in this paper.

2) The method used for testing the correlation coefficients is not given. Most probably they have used Fisher Z-test, but it is several other methods available. Fisher Z-test is shown to be the overall best test in such situations.

3) As long as they want to use correlation coefficients in the presentation of the results, 95% confidence intervals are recommended

Results:
1) “Patients” given under results is a description of the material and is already given. It has to be taken out from the result chapter. In general, description of the material is recommended to be given as results only in epidemiological studies. I will classify these studies as controlled clinical trials and the description of the sample shall therefore be given under “Material & Methods”.

2) The results given in Table 5 are repeated in the text. Results have to be given either in tables / figures or in the text. Double reporting of results shall be omitted.

3) The estimated correlation coefficients with 95% confidence intervals are lacking in the results. These coefficients are given in the figures, but the figures express simple linear regression. If additional results are wanted be given in these figure, the regression coefficient or the regression line (Y=#0+#1X) can be written.

Tables and figures:
1) Table 1 and 2 should be omitted and replaced by references in the text.

2) In table 4, “Median CAI” and “Median EAI are given, but in text under the table it is stated that the results are give as means? Are the numbers given in the table Median or Mean values? Please describe that under “statistical analysis”. If Median is given, describe why? In the correlation analysis and the simple linear regression analysis (figures), the mean is the central estimator.

3) The results given in table 5 are nearly covered by the results described in the text. Add the missing information in the text and omit table 5.

4) Take out the correlation coefficients from the figures and replace that with the estimated linear regression line as previously described.

Stig Larsen MD, PhD Medical Statistics
Professor in Controlled Clinical Research Methodology and Statistics
Norwegian School of Veterinary Science & Ullevål University Hospital