Reviewer's report

**Title:** Cytokine gene polymorphisms and serum cytokine levels in patients with idiopathic pulmonary fibrosis

**Version:** 1  **Date:** 27 November 2012

**Reviewer:** Grethe Neumann Andersen

**Reviewer's report:**

The manuscript describes findings in an appreciable cohort of patients with a severe, but rather unusual pulmonary condition: idiopathic pulmonary fibrosis. The methods are sound and the results worth publishing. However, the way the results are presented in tables and figures should be improved considerably, the discussion condensed and the number of references diminished.

**Major considerations:**

1. The discussion is too long and should be appreciably condensed.

   The possibility that IL-10 may be produced by regulatory T cells should be discussed.

   Moreover, the frequencies of the gene polymorphisms (IL-6 -174 and TNF# -308) have been examined by Pantelidis et al in a British population. The results as to the gene polymorphisms presented herein should be discussed in relation to results from similar studies in other populations.

2. The tables should be improved,

   In Table 1 the characteristics of the healthy controls should be incorporated, that is their number, sex, age and smoking habits.

   Table 2 is difficult to comprehend and needs a major revision, maybe it should be split into 2 or 3 minor tables. In order to make the table more informative and clear, the allele frequencies in the 2 populations (IPF and healthy controls) should be calculated and shown.

   Especially the data on the gene polymorphisms in the promoter region of IL-10 are difficult to understand.

   For example you have the first genotype (-1082, -819 and -592):

   GCC GCC

   and the second:

   GCC ACC

   This could also be written as below, where IL-10 1 stands for haplotype 1.

   -1082 -819 -592
IL-10 1 GCC GG CC CC
IL-10 2 ACC GA CC CC
IL-10 3 ATA GA CT CA
IL-10 4 ACC AA CC CC
AA CT CA
AA TT AA

I guess this means that of 8 possible haplotypes, (given 3 loci with 2 possible alleles each = 2 x 2 x 2 = 23), only 4 are represented in the Saudi population and 6 combinations of these?

An example of how to show haplotype and allele frequencies in a comprehensible manner can be found in your reference number 5 (Pantelidis et al Respir Crit Care Med 2001;163:1432-6) table 1 and 2.

Moreover, when I calculate the allele frequencies from your table, I find that the frequencies for the C and T alleles at the IL-10 (-819) locus are equal to frequencies for the C and A alleles at the IL-10 (-592) locus. Is this correct or is it an error?

Allele frequencies are calculated thus: -you simply add the number of alleles found in homozygotes and heterozygotes-

For example for TNF# (-308) in IPF (n = 60 with 2 alleles each = 120 alleles):
Frequency of allele A = 4 x 2 + 19 x 1 = 27 alleles out of 60 x 2 = 27/120 x 100% = 22.5%.
Frequency of allele C = 19 x 1 + 37 x 2 = 93 alleles out of 120 = 93/120 x 100% = 77.5%

As to the TGF#1 polymorphisms- you may present these data in the same way as the data on the IL-10 haplotypes, that is
Codon 10 Codon 25
TGF#1 haplotype1 TG TT GG
TGF#1 haplotype 2 CG TC GG
TGF#1 haplotype 3 CC TC GC
TGF#1 haplotype 4 TC (not found) TT GC

You may omit the TC haplotype, which you did not find and mention this in the table text. Also here you should calculate allele frequencies.

3. It would be nice if you could present the results from your ELISA analysis as a figure, for example as Box plots with 25 and 75 percentiles. However, I understand that many controls had IL-6 levels that were not measurable. However, you may choose to show these as the cut of value or a value just below the cut of.
Minor considerations:

In the section on background it is said in line 2 that fibrosing interstitial pneumonia is limited to the lungs. You should omit the phrase “limited to the lungs” as it is obvious that lung diseases are limited to the lungs.

In the same section, line 11, the serum levels of IL-6 and TGF####(the word serum should be added).

In the part with the heading: Chest CT: I suppose you mean high resolution (HRCT) scans.

The number of references should be cut down to 30. The references should be edited according to international standards.

**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:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

'I declare that I have no competing interests'