Format for ANSWERING REVIEWERS

April 5, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: ESPS 2371-Review.doc).

Title: Cytokine gene polymorphisms in idiopathic pulmonary fibrosis

Author: Martina Vasakova, Martina Sterclova, Libor Kolesar, Antonij Slavcev, Jelena Skibova, Martina Langova, Ilja Striz

Name of Journal: *World Journal of Respirology*

ESPS Manuscript NO: 2371

The manuscript has been improved according to the suggestions of reviewers:
1Format has been updated to a Brief communication.
2Revision has been made according to the suggestions of the reviewer

(1) R1: ? However, the discussion for the mechanism is a little bit weak. **Answer:** The discussion was improved.

(2) R2: ? Although previously published findings should be mentioned, please provide only relevant recent findings and avoid repeating previously published findings. These manuscript seems to describe the same findings previously published (Cytokine gene polymorphisms and high-resolution-computed tomography score in idiopathic pulmonary fibrosis.Vasakova M, Striz I, Dutka J, Slavcev A, Jandova S, Kolesar L, Sulc J. Respir Med. 2007 May;101(5):944-50. Epub 2006 Oct 23. PMID:17056243) **Answer:** I have avoided mentioning previous findings in detail. The current manuscript does not describe the same data. We present the data of extended group of patients, which increases the statistical strength of the results compared to previous study on genetic polymorphisms in IPF published in Tissue Antigens 2006 and in the study on genetic polymorphisms and HRCT findings which you have mentioned.

(3) R3: ? there are several orthographical and grammar errors (e.g. page 8 "differencies" should read "differences"). Thus, the authors should consider seeking advice by a native speaker. **Answer:** I appologize for the errors, the manuscprit now has been finally checked by recommended agency.

? Is the Il-6 polymorphism also functional? **Answer:** Yes, it is. The IL-6 (-174) G→ C polymorphism was proved to code higher IL-6 production. It is has been stated in the Discussion.

? The statistics should be corrected for multiple analyses. **Answer:** I do agree, it has been corrected, and IL-6 results then has fallen below statistical significance. It has been stated in the Discussion.

? I agree that IPF/UIP might be more common in male, however, the more doubtful is the comparison with a control group containing only 20% male! In general, genetic association

studies normally require large cohorts. I know that it is hard to get enough patients for such studies in rare lung diseases, especially in monocentric studies. Thus, the authors should give a caveat on their small patient groups in the discussion. **Answer:** We have given the caveat on it in the Discussion.

? do all patients present with a pattern consistent with UIP? The high numbers of lymphocytes are more consistent with NSIP. **Answer:** Yes, they did, and also other potential causes of ILD with UIP HRCT pattern were excluded. I have added it to the Methods.

? Several p values are given as 0.000, e.g. page 3, last but second line, page 8, first 2 paras of the Results section, **Answer:** I have corrected it.

? The term "fibroproduction" is not clear to me ? **Answer:** I have changed it to fibroproliferation.

? I never heard the term "mendelian population". There might be a mendelian inheritance of certain genes. I assume that you want to explain that the Czech population does not root from a single founder population with similar genetic background – as it is for most of the European populations. **Answer:** Thank you for better formulation. I have corrected it accordingly.

? Page 9: CVID is the common variable >immune< deficiency ? **Answer:** Yes. I have corrected it

? Page 9, Discussion: the sentence starting with "According to the direct role of IL-4 in the pathogenesis of fibrosis, in vitro studies...." Needs some citastions. **Answer:** Citations have been added.

? Brevity of result section: The result section on page 8 is short and the only new data presented is on the differences in IL4 and IL6 promotor region polymorphisms in the IPF and control population. This result in isolation however provides no direct evidence to support the conclusion of the authors that:"We have suppported our hypothesis about a role of IL-4 gene promotor polymorphisms, which can cause a shift to Th2 type immune response, in IPF pathogenesis." The authors should state more carefully that the significance of this observation in terms of IPF pathogenesis and in terms of clinical implications would need to clarified in future studies. And given the brevity of the reported data this manuscript could be better suited as a "brief communication". **Answer:** I have rearranged the Results section. The manuscript was re-written as a Brief communication. The statements in Conclusions were modified accordingly.

? It would be very helpful if the authors could expnad their results in this investigation to provide evidence of the observed differences in IL4 and IL6 polymorphisms to IPF pathogenesis and clinical progression: For example could the authors expand their results to include information on: 1. Were there differences in IL-4 and IL-6 levels? 2. Were there clinical differences in the 55% IPF patients with the IL-4 -590 CT polymorphism compared to the 42.9% with the CC genotype? a) Clinical differences in terms of disease progression (decline in FVC, high-resolution-computed tomography fibrosis scores, mortality or need for lung transplant) b) Clinical differences in the occurrence of IPF exacerbations. **Answer:** This study deals only with polymorphisms frequency. Thus it was also upon recommendation of reviewers changed to a Brief communication. We did not measure the values of IL-4 and IL-6, it is a subject for our currently running study on BALF proteomic in IPF. Clinical data were not complete in all of the patients (i.e. not all had serial HRCT, some of the patients are still living, they have different treatment), so we did not evaluate it in

this study, but it will be a subject for further study as well.

? The result section in the abstract is longer then in the main text body. **Answer:** I have redone it.

(4) R4: ? Therefore, the complexity of the process may prevent its explanation by the merely involvement of a particular group of cytokines. Also, the possibility of synergisms or antagonisms among different cytokines, make it difficult to study this process without analyzing the global interaction between them. However, I believe it is important the realization of studies such as the one submitted, in order to try and add "pieces" in this complex "puzzle". This comment, or a similar one, could be added in the discussion. **Answer:** It was added to the Discussion.

? Population: a) The groups are NOT comparable in age or gender, and, although the authors indicate the latter as one of the study's weakness, it might influence the results. Being a prospective study, I am struck by the choice of the control group. Anyways, in my opinion, this DOES NOT invalidates the possibility of accepting the article for publication. If possible, it should be argued based on the literature, that the influence of age and gender in the cytokine levels obtained, doesn't invalidate the conclusions. **Answer:** If we consider cytokine values, they could be influenced by age and tender, but we did not find any data in literature dealing with the influence of age and gender on cytokine genotype. We suppose, that sex and age possibly does not influence genotype of cytokines. It was added to the Discussion.

? In the control group, it should be indicated, in an explicit manner, if the subjects HAVE/DON'T HAVE asthmatic affection or systemic inflammatory disease. **Answer:** We have added this information to the Methods.

? Although the number of subjects with IPF is high (56), accounting for the difficulties in recruitment of this patients, properly designed multicentric studies, with a higher number of subjects might be in order, to achieve higher statistical power and obtain relevant conclusions. **Answer:** Yes, I agree. A multicentric, study on genetic polymorphisms in IPF is needed to support our findings. I have stated it in the Discussion.

? The amount of patients with biopsy, obtained by video Thoracoscopy, is "low" (16/56). However, this could be explained by the mean age of the subjects (67 years old), where most of them would not be candidates for lung transplant, one of the most frequent indications for such technique. If these were the case, it could be commented in the discussion. **Answer:** Surgical lung biopsy is not required for the diagnosis of IPF (even not in potential lung transplant recipients) in the cases which are clear from the clinical and radiological point of view, especially in advanced stages of the disease and/or in the elderly. That´s why we have stated in the Methods that we performed surgical lung biopsy only in the cases who did not meet clinical and radiological criteria for IPF, which we believe might be sufficient.

? Results: Given the current believe that an structural change in IPF exists (uncontrolled fibroblast proliferation), and not just an inflammatory process, I find the results obtained somehow odd. Something regarding this could be commented in the discussion. **Answer:** The results have been commented in the Discussion accordingly.

? Discussion: It is properly organized. It could be modified in part, by adding updated bibliographic references (See "References"). **Answer:** The discussion has been modified according to added references.

? References: The latest reference is from 2010, and there are none from the past two years.

However, the subject in matter (cytokines and pulmonary fibrosis, or interstitial lung disease) has many new publications in the latest years. It should be updated. **Answer:** New references has been added.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Respirology*.

Sincerely yours,

Martina Vasakova, MD, PhD, Assoc. Prof.
Dept. of Respiratory Medicine, 1st Medical School, Charles University
Thomayer Hospital
Videnska 800
140 59 Prague 4
Fax: +420 261082206
E-mail: martina.vasakova@ftn.cz