Author's response to reviews

Title: High Serum Concentrations of Autoantibodies to HSP47 in Nonspecific Interstitial Pneumonia Compared with Idiopathic Pulmonary Fibrosis

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BMC Pulmonary Medicine
Dear Executive Editors,

Thank you for your e-mail of 07-May-2008. We were pleased to know of your positive evaluation of our manuscript and its potential acceptance for publication in the BMC Pulmonary Medicine, subject to adequate revision and response to the reviewers’ comments.

We take this opportunity to express our gratitude to the reviewers for their constructive and useful remarks. Their comments allowed us to identify areas in our manuscript that needed modification and clarification. We also thank you for allowing us to resubmit a revised copy of the manuscript.

I hope that the revised manuscript is now acceptable for publication in the BMC Pulmonary Medicine.
Response to the comments of Reviewer 1

We thank the reviewer for the positive evaluation of our manuscript and the pertinent and important comments.

(1-3) We thank the reviewer for the pertinent and important comments. This is a retrospective study. There was no intended selection because the subjects of this study were all the patients admitted to our institution from April 1997 to March 2004 and diagnosed pathologically as interstitial pneumonia. However, the patients groups might not represent general patient population with IIPs because the diagnosis of UIP and NSIP was confirmed pathologically by open lung biopsy or video-assisted thoracoscopic surgery and patients diagnosed only clinically were excluded. Accordingly, the study population may represent only “early and moderate” stage of ILD. As pointed by the referee, our study included only a small number of patients and the study population may represent “early and moderate” stage of ILD. In order to confirm the results of this study, well-planned prospective study using a large number of patients are required. As advised by the reviewer, we added above information in Method and Discussion section (page 5, para 1, lines 3-6, page 9, para 2, lines 10-21).

(4) We accept the criticism made by the reviewer that we should examine and describe other laboratory tests including biochemical and serological tests such as rheumatoid factors and etc. in healthy volunteers. Unfortunately, we did not examine them. Healthy volunteers included 8 male and 10 female, and their median age was 31 (range 26-60). As the reviewer pointed out, age of healthy volunteers did not match with that of patients with IIPs.

(5) We accept the criticism made by the reviewer that we did not show the mechanism of elevated serum levels of HSP47 autoantibody in fibrosing NSIP. As we previously reported, expression of HSP47 was noted in fibroblasts, myofibroblasts and type II pneumocytes in idiopathic interstitial pneumonia [17, 18]. The expression level of HSP47 in type II pneumocytes of idiopathic UIP was significantly higher than that in idiopathic NSIP [18]. In contrast to that, in this study, we demonstrated that the anti-HSP47 titers of patients with idiopathic NSIP were significantly higher than those of patients with idiopathic UIP. We speculate that HSP47 autoantibody might neutralize the HSP47 antigen and
suppress the fibrosis in idiopathic NSIP. However, there is no direct evidence. Further studies are warranted in order to elucidate the precise mechanisms.

We added the above information in Discussion section (page 11, para 2, lines 11-19). In addition to that, many of the patients with idiopathic NSIP develop collagen vascular disease later on in the disease course. The fact that the serum levels of autoantibodies to HSP47 in patients with idiopathic NSIP were significantly higher than in patients with other IIPs suggests that the patients with so called “idiopathic” NSIP might display signs of auto-immunity in contrast to patients with idiopathic UIP. We discussed this point in Discussion section (page 11, para 1, lines 6-10). We also already described the possible mechanisms in Discussion section (page 10, para 3, line 11- page 11, para 1, line 6).

(6) We previously reported a lot of pathological and physiological data with regard to increase in production or decrease in metabolism and excretion of HSP47. HSP47 is a collagen-binding, stress-inducible protein localized in the endoplasmic reticulum and is never released into the extracellular matrix. HSP47 has a specific role only in the intracellular processing of procollagen production as a collagen-specific molecular chaperone [9-12]. HSP47 expression is upregulated in animals with experimentally-induced fibrosis, including murine bleomycin-induced pulmonary fibrosis [13, 14], rat peritoneal sclerosis [15] and carbon tetrachloride-induced rat liver cirrhosis [16]. In addition, we reported previously that there was also increased expression of human HSP47 in the fibrotic lesions of idiopathic pulmonary fibrosis (IPF) [17, 18], fibrotic transplanted kidney [19], and peritoneal sclerosis [20]. Recent reports have demonstrated that HSP47 expression is highly tissue- and cell-specific, restricted to mostly phenotypically altered collagen-producing cells, and correlates well with that of collagen [13, 17-20]. These findings suggest the important role of HSP47 in collagen synthesis in various fibrotic disorders. HSP47 is also identified as an autoantigen in the sera of several rheumatoid arthritis (RA) patients [21, 22]. Higher levels of HSP47 protein and autoantibodies to HSP47 in sera were also found in patients with the rheumatic autoimmune diseases, especially mixed connective tissue disease (MCTD) [23]. We have already described above information in Background section (page 3, para 3, line 20- page 4, para 2, line 15).

Response to the comments of Reviewer 2

We thank the reviewer for the positive evaluation of our manuscript and the pertinent and important comments.

(1) We accept the criticism made by the reviewer that the main hypothesis of the article that it might be possible to discriminate NSIP from other IIP by the measurement of serum HSP47 autoantibody is hazardous. Accordingly, we re-wrote the manuscript regarding this subject in Abstract, Background and Discussion section. We also deleted some sentences. We changed the contents widely. In addition to that, we gave a scattergram to show the power of discrimination between the distinct disease groups (Figure 1, 2).
(2) We understand the criticism made by the reviewer that how the authors could include a similar amount of patients with UIP/IPF and NSIP in this study. The subjects of this study were all the patients admitted to our institution from April 1997 to March 2004 in whom the diagnosis of interstitial pneumonia was confirmed pathologically. The diagnosis of UIP and NSIP was confirmed pathologically by open lung biopsy or video-assisted thoracoscopic surgery and patients diagnosed only clinically were excluded. Recently, clinical diagnostic criteria of IPF has been established, so surgical lung biopsy is seldom performed in typical IPF patients. That is why we could include a similar amount of patients with UIP and NSIP. There was no intended selection. We added the above information in Method section (page 5, para 1, lines 3-6). We also agree with comment made by the reviewer that many of the patients with idiopathic NSIP will develop collagen vascular disease later on in the disease course. The fact that the serum levels of autoantibodies to HSP47 in patients with idiopathic NSIP were significantly higher than in patients with other IIPs suggests that the patients with so called “idiopathic” NSIP might display signs of auto-immunity in contrast to patients with idiopathic UIP. We discussed this point in Discussion section (page 11, para 1, lines 6-10).

(3) We agree with the comment made by the reviewer that it would be highly interesting to show whether HSP47 autoantibodies are neutralizing HSP47 antigen in the fibrosing lung. We speculate that HSP47 autoantibody might neutralize the HSP47 antigen and suppress the fibrosis in idiopathic NSIP. However, there is no direct evidence. Further studies are warranted in order to elucidate the precise mechanisms. We added this comment in Discussion section (page 11, para 2, lines 14-19).

(4) The diagnosis of NSIP was confirmed pathologically and classified according to the American Thoracic Society/European Respiratory Society consensus criteria [1]. Idiopathic NSIP patients was classified into 2 groups: cellular and fibrosing pattern (Group 2) and fibrosing pattern (Group 3). The pathological diagnosis was established by Dr. M Kitaichi (Department of Laboratory Medicine and Pathology, NHO Kinki-chuo Chest Medical Center) who are a core panel member of “American Thoracic Society/European Respiratory Society International Multidisciplinary Consensus Classification of the Idiopathic Interstitial Pneumonias” statement [1]. We accept the criticism made by the reviewer that it is difficult for the reader to discriminate between group 2 and group 3. Accordingly, we classified “cellular and fibrosing” and “fibrosing” instead of “group 2” and “group 3” in the revised manuscript.