Author's response to reviews

Title: Myositis autoantibodies in Korean patients with inflammatory myositis: Anti-140-kDa polypeptide antibody is primarily associated with rapidly progressive interstitial lung disease independent of clinically amyopathic dermatomyositis.

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Author's response to reviews: see over
August 15th, 2010

Dear Editor,

Thank you for your e-mail of August 10th, 2010 regarding our manuscript (MS: 1232647947426393), together with the comments from the reviewers. The manuscript has been revised as follows to address the issues raised by the reviewer. The changes were highlighted with red-colored text.

**Reply to reviewer 1.**

1. The anti-p140 autoantibodies as published are seen most frequently in CADM. Thus referring to them as “myositis-specific autoantibodies” or MSA is not appropriate since most of the published cases do not have clinical myositis. Also, the inappropriate use of the term MSA - which has been reserved for those autoantibodies that have been extensively studied in myositis and non-myositis populations to assure their specificity – by other groups describing anti-p140 or anti-p155/140 as such, is not an excuse to continue this error. A more accurate description of the anti-p140 and anti-p155/140 autoantibodies would be “autoantibodies frequently seen in myositis” or better yet “myositis autoantibodies” and I believe this term should be used throughout the paper and the point should be made in the discussion about the inappropriate use of the term “MSA” in other papers.

→ We understand the issue raised by the reviewer regarding the appropriate usage of the term “MSA” and agree that anti-p140 and anti-p155/140 antibodies have not been extensively studied in non-myositis populations to assure their specificity for myositis. We modified the term so that anti-p140 and anti-p155/140 antibodies are not referred to as MSAs in this paper. In addition, we described that anti-p140 and anti-p155/140 antibodies are yet to be studied more extensively in non-myositis patients before referring to them as “MSAs” in the introduction section (we added this comment in the introduction rather than in the discussion because this issue is not directly related to the purpose/results of the present study).

**TITLE:** Myositis autoantibodies in Korean patients with inflammatory myositis: Anti-140-kDa polypeptide antibody is primarily associated with rapidly progressive
interstitial lung disease independent of clinically amyopathic dermatomyositis.

(Page 3, line 2) “To investigate the association between myositis autoantibodies and clinical subsets of inflammatory myositis in Korean patients.”

(Page 3, line 6) “A panel of defined myositis autoantibodies was surveyed to investigate the association between each autoantibody and clinical subsets of inflammatory myositis.”

(Page 3, line 8) “Either MSAs, anti-p140, or anti-p155/140 antibodies were found in 63.3% (31/49) of the study subjects.”

(Page 3, line 11) “All MSAs and anti-p140 and anti-p155/140 antibodies were mutually exclusive.”

(Page 4, line 2) “Anti-p140 and anti-p155/140 antibodies were commonly found autoantibodies in Korean patients with inflammatory myositis.”

(Page 6, line 3) “In recent years, novel autoantibodies have been identified in inflammatory myositis, such as, anti-140-kDa polypeptide (anti-p140) [9] and anti-155/140-kDa polypeptide (anti-p155/140) antibodies [10, 11]. Because these antibodies have yet to be extensively studied in non-myositis populations to assure their specificity for myositis and because the presence of anti-p140 antibodies has been largely limited to CADM patients who do not have clinical muscle symptoms [9, 12, 13], it may be currently inappropriate to classify anti-p140 and anti-p155/140 antibodies as MSAs. However, associations between these novel antibodies and distinctive clinical subsets have been found in adult inflammatory myositis patients; associations between anti-p140 antibody and CADM-associated ILD [9, 12, 13] and between anti-p155/140 antibody and cancer-associated myositis are such examples [10-12, 14-16].”

(Page 6, line 17) “In the present study, we investigated the panel of defined autoantibodies including MSAs, MAAs, anti-p140, and anti-p155/140 antibodies in
the sera of Korean inflammatory myositis patients with the intention to classify clinical subsets of these patients based on the presences of myositis autoantibodies and to refine the relationships between these antibodies and disease manifestations.”

(Page 11, line 2) “The MSAs, anti-p140, and anti-p155/140 antibodies were found to be mutually exclusive.”

(Page 11, line 9) “We then examined whether any associations existed between the myositis autoantibodies and the clinical features of myositis.”

(Page 12, line 13) “The present study shows that anti-p140 (18.4%), anti-p155/140 (16.3%), anti-Mi2 (14.3%), and anti-ARS (12.2%) antibodies are common autoantibodies in Korean patients with inflammatory myositis.”

(Page 14, line 1) “On the other hand, our study shows that anti-p140 antibody is one of the most common myositis autoantibodies in Korean patients with classic DM, and that it has a striking association with rapidly progressive ILD.”

(Page 14, line 17) “In fact, cancer-associated myositis in the presence of other myositis autoantibodies than anti-p155/140 antibodies (such as anti-ARS or anti-Mi2) has been previously reported [14, 27, 28]. Although anti-p140 positive patients have been shown to have low prevalence of malignancy [9, 12], the presence of other myositis autoantibodies rather than anti-p155/140 antibodies does not seem to rule out the presence of cancer in inflammatory myositis patients.”

(Page 15, line 8) “Second, we did not further examine the specificities of myositis autoantibodies beyond immunoprecipitation.”

(Page 15, line 13) “In addition, distinctive clinical features have been demonstrated in association with each myositis autoantibody defined by immunoprecipitation in our study, which is generally in parallel with the results of previous studies [9-16].”

(Page 16, line 1) “In summary, anti-p140 and anti-p155/140 antibodies were
commonly found autoantibodies in Korean patients with inflammatory myositis.”

2. As shown in Figure 1B, some of the patients assigned as having anti-p155/140 or anti-p140 appear to have other IP bands that make their designation uncertain (also other patients, such as D, have IP bands in this region that apparently were not called as having these autoantibodies). The general experience in the field is that immunoprecipitation alone is often misleading in the identification of these autoantibodies. For this reason, because of the large number of possible protein bands in this region, many investigators do not believe that protein immunoprecipitation alone can define with certainty the anti-p155/140 reactivity and distinguish it from the anti-p140 or other reactivities, and that IP followed by immunoblotting or another validated method is needed. The authors have not confirmed these reactivities as other groups have, but rather appear to be saying that they are using an operational definition of these autoantibodies as: anti-p140 positive = a strong immunoprecipitation of a 140 KD protein that matches the prototype serum; and anti-p155/140 positive = strong immunoprecipitation of 155KD and 140KD proteins that match the prototype serum. If so, then the authors need to explicitly define their reactivities this way and add an additional sentence in the discussion that other groups have used other techniques to confirm these reactivities that may result in different results from theirs.

We explicitly described in the method section how we defined the presence of these antibodies, and added an additional sentence in the discussion about this issue.

(Page 9, line 12) “The presence of anti-p140 or anti-p155/140 antibodies was defined when apparent protein precipitates were found either at 140-kDa (anti-p140) or at 155/140-kDa (anti-p155/140) which match the reference sera. Studies to further confirm the specificities of these antibodies were not performed.”

(Page 15, line 9) “Therefore, anti-p140 and anti-p155/140 antibodies detected in the present study may not exactly represent anti-CADM-140 and anti-p155/140 antibodies that have previously been shown to recognize MDA5 [13, 26] and transcriptional intermediary factor 1-γ [29], respectively.”
Reply to reviewer 2.

1. When the authors report no association of ARS positivity with malignancy they compare 3/6 to 8/43. I recognize why the PL-7 (+) pt does not count (i.e. cancer detected 48 months prior to cancer) but is it correct to use the 8/43 ratio given the fact that the 155/140 (+) patients are included in this ratio?

To examine the association between anti-ARS and malignancy in myositis patients, we performed a univariate analysis on the following 2x2 table using chi-square test (that is, compared the frequency of cancer-associated myositis in anti-ARS positive patients versus the frequency of cancer-associated myositis in anti-ARS negative patients). The P value obtained was insignificant (p = 0.117).

<table>
<thead>
<tr>
<th></th>
<th>Anti-ARS +</th>
<th>Anti-ARS -</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer –associated myositis +</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>Cancer –associated myositis -</td>
<td>3</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>43</td>
</tr>
</tbody>
</table>

The association of anti-ARS with cancer-associated myositis independently of anti-p155/140 may be obtained using multivariate analysis, which, however, seems to be un-necessary because anti-ARS and anti-p155/140 antibodies were mutually exclusively found in myositis patients.

2. How do the authors get the specificity of 85.3% for the association of anti-p140 with rapidly progressive ILD?

Because anti-p140 antibody was exclusively found in DM patients, the specificity was calculated within DM group. Based on the following table, the specificity for the association with rapidly progressive ILD is 85.3% (29/34).

<table>
<thead>
<tr>
<th></th>
<th>Anti-p140+</th>
<th>Anti-p140-</th>
</tr>
</thead>
<tbody>
<tr>
<td>rapidly progressive ILD +</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>rapidly progressive ILD -</td>
<td>5</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>29</td>
</tr>
</tbody>
</table>

We modified the related text as follows to clearly describe that the specificity was
calculated within DM patients.

(Page 3, line 13) “Anti-p140 antibody was associated with rapidly progressive interstitial lung disease (ILD) (p = 0.001), with a sensitivity of 100.0% and a specificity of 85.3% in DM patients”

Reply to reviewer 3.

1. The wording concerning the reasons for not using anti-CADM-140 rather than anti-p140 could be improved [Second, we did not further examine the specificity of each MSA beyond immunoprecipitation, which let us reserve the term “anti-CADM-140 antibody”] would be better phrased as ... which dissuaded us from using the term anti-CADM-140.

→ We would be happy to modify the text as the reviewer 3 suggested. However, the issue #2 raised by the reviewer 1 also involves this part of the manuscript and the modified text amounts as follows.

(Page 15, line 8) “Second, we did not further examine the specificities of myositis autoantibodies beyond immunoprecipitation. Therefore, anti-p140 and anti-p155/140 antibodies detected in the present study may not exactly represent anti-CADM-140 and anti-p155/140 antibodies that have previously been shown to recognize MDA5 [13, 26] and transcriptional intermediary factor 1-γ [29], respectively.”

2. It would still be important to cite the study of anti-p155/140 reported by Gunawardena et al Rheum 2008 47;324-328 that includes original data on adult myositis, cancer and anti-p155/140 - and may also help answer other reviewer comments.

→ We agree that the study by Gunawardena et al presents significant original data on the association between cancer and anti-p155/140 antibody in adult myositis, and cited this study (reference #16 of the revised manuscript).