Author's response to reviews

Title: Anti-centromere antibody-seropositive Sjogren's syndrome differs from conventional subgroup in clinical and pathological study

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Version: 2 Date: 19 May 2010

Author's response to reviews: see over
Dear Editor,

May 19, 2010

We submit our revised manuscript entitled “Anti-centromere antibody-seropositive Sjögren’s syndrome differs from conventional subgroup in clinical and pathological study” by Nakamura H et al. We revised our manuscript according to the reviewers’ comments point to point. We added sufficient number of anti-centromere antibody (ACA)-seronegative Sjögren’s syndrome patients to determine the characteristics of ACA-seropositive SS patients with supportive background data. The manuscript has been approved by all the authors and they have given necessary attention to the integrity of the work by their critical reading.

We hope that our revised manuscript will be acceptable for publication in BMC Musculoskeletal Disorders.

Yours sincerely,

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Reviewer's report

Title: Anti-centromere antibody-seropositive Sjogren's syndrome differs from conventional subgroup in clinical and pathological study

Version: 1 Date: 1 April 2010
Reviewer: Dr. Yves RENAUDINEAU

Reviewer's report:
Nakamura and colleagues present new data regarding the characterization of a subgroup of patients with primary Sjögren’s syndrome (pSS) positive for anti-centromere antibodies (ACA). To test this hypothesis, the authors test the amount of collagenous fibres, in minor salivary glands, and observed differences between ACA+ and ACA- subgroups.

Major Revisions
1- The prevalence of ACA-seropositive patients among pSS patients is not provided.

Reply
Since we calculated the prevalence of ACA+ pSS in all pSS patients in our department, we added next sentences.

Page 3, line 57
Abstract
Results:
Prevalence of ACA+SS patients was 14 out of 129 (10.85%) pSS patients.

Page 7, line 146
In our institution, the prevalence of ACA+ pSS patients was 14 out of 129 (10.85%) pSS patients who strictly met AECG classification criteria in our medical records, although the entire 129 pSS patients were listed with or without examination for labial salivary biopsy.

Furthermore, we added related discussion as follows;
Page 8, line 181
With regard to the prevalence of ACA in pSS patients, Salliot C et al (13) previously described as 4.7% in 212 pSS patients in France. Prevalence in our data (10.85%) was
statistically higher than that of their data ($p=0.032$ by chi-square test) despite the same measurement of antibodies against CENP-B. It might derive from genetic background or difference of ELISA kit.


2- The sample size of the ACA-seronegative group should be increased, at least 50 patients have to be included (3 times the ACA-seropositive population for statistical reasons).

**Reply**

We selected 48 ACA- pSS patients with minor salivary gland biopsy. We updated background data including follow-up period in results/Table 1 and calculated fibrotic area by Azan staining in results/Fig 2. The amount of fibrous tissue was $26251.3 \pm 14249.8 \mu m^2$ in the 48 ACA- pSS patients with significance ($p=1.3 \times 10^{-12}$).

Updated background data was shown in Table 1 as follows:

<table>
<thead>
<tr>
<th></th>
<th>ACA (+)</th>
<th>ACA (-)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (M/F)</td>
<td>14 (0/14)</td>
<td>48 (1/47)</td>
<td>0.59</td>
</tr>
<tr>
<td>Age</td>
<td>57.4 ± 9.6</td>
<td>58.3 ± 13.2</td>
<td>0.82</td>
</tr>
<tr>
<td>Follow-up period (year)</td>
<td>6.6 ± 5.6</td>
<td>4.5 ± 4.6</td>
<td>0.16</td>
</tr>
<tr>
<td>Raynaud’s phenomenon</td>
<td>8/13 (61.5%)</td>
<td>4/48 (8.3%)</td>
<td>1.86x10^{-5}</td>
</tr>
<tr>
<td>anti-SS-A/Ro Ab or anti-SS-B/La Ab</td>
<td>0/14 (0.0%)</td>
<td>37/48 (77.1%)</td>
<td>2.30x10^{-7}</td>
</tr>
<tr>
<td>IgG (mg/dl)</td>
<td>1530.2 ± 267.1</td>
<td>2056.0 ± 730.2</td>
<td>0.018</td>
</tr>
<tr>
<td>Average of FS</td>
<td>1.4 ± 1.0</td>
<td>2.3 ± 1.6</td>
<td>0.035</td>
</tr>
</tbody>
</table>
P value for the prevalence of RP and IgG was less than 0.05.

3- To compare biopsy gradations between groups, the duration of the disease should be indicated as well as the medications in table I.

**Reply**
As we updated in the above table, there was no statistical difference in follow-up period.

We also added next sentence in results (Page 7, line 153)
There was no statistical difference in follow-up period from point of diagnosis between ACA+ and ACA- pSS groups.

**Minor Revisions**
4- Only sclerodactyly is used to exclude scleroderma. Other criteria should be provided in order to exclude clearly the scleroderma patients with secondary SS. Ideally a scleroderma population with secondary SS may be included, if not done this point should be discuss.

**Reply**
According your comments, we added next sentences in the last part of discussion. Page 10, line 212

Avouac J et al (16) also previously reported that the prevalence of secondary SS was 14% and was strongly associated with limited cutaneous SSc. Although we excluded limited cutaneous SSc in the present study according to the criteria determined by LeRoy et al (17), SSc complicated with secondary SS should be carefully examined during follow-up period.

We also added related references 16 and 17 as follows;
5- It is stated that IgG are reduced in the ACA-seropositive subgroup. However, since the ACA-seropositive subgroup is not different from the normal range, the good interpretation is that an hypergammaglobulinemia characterize the ACA-/SS subgroup but not the ACA+/SS subgroup which is normal, as recently described [Bournia, 2010]. Please correct accordingly.

Reply
Thank you for your kind advice for IgG in ACA+ and ACA- subgroups. According to your comments, we changed “low” to “normal” with regard to IgG in ACA- pSS subgroup and also added next sentences in Discussion.

Page 9, line 205
More recently, Bournia VK et al (15) reported that hypergammaglobulinemia was characteristic of not ACA+ pSS but ACA-SS subgroup, which suggests ACA+ pSS subgroup forms intermediate entity between ACA- pSS and SSc.

6- Material section, the technique used to measure IgG is not specified. The utilisation of the abbreviations is not rigorous please correct (examples: seropositive/+/positive, non ACA/conventional/negative, pSS/SS…).

Reply
Regarding the measurement for IgG, we added next sentence.
Page 5, line 107
Serum IgG concentration was measured by a nephelometric immunoassay.

We also integrated the term of patients groups as ACA+ pSS and ACA- pSS in the text.

In conclusion: fibrous analysis results are promising but such data needs to be clearly demonstrated by increasing the patients in the control group (SS without ACA and may be also scleroderma patients with secondary SS).

Reply
We increased the number of ACA- pSS patients to 48 and re-calculated background data and fibrotic area determined by Azan staining.
Reviewer's report
Title: Anti-centromere antibody-seropositive Sjogren's syndrome differs from conventional subgroup in clinical and pathological study
Version: 1 Date: 5 April 2010
Reviewer: Dr. Wan-Fai Ng

Reviewer's report:
Major Compulsory Revision
1. Since all the ACA+ patients are negative for anti-Ro/La antibodies while 80% of the comparison group of ACA- patients are positive for anti-Ro/La antibodies, have the authors investigated whether the observed difference between the ACA+ and ACA- groups simply or at least partially reflecting the difference between Ro/La+ versus Ro/La- primary Sjogren's syndrome? Using an additional Ro/La negative pSS group for comparison will be needed to address the above.

Reply
Thank you for your comments on the number of ACA- pSS subgroup. According to your suggestion, we increased the number of ACA- pSS patients from 10 to 48 and analyzed background data and fibrotic area.

We also added next negative data regarding sicca in results.

Page 7, line 155
ACA- patients showed either xerophthalmia (56.3%) or xerostomia (72.9%) with 85.7% positivity in the Saxon test. In addition, these 3 parameters showed no statistical significance between ACA- and ACA+ pSS patients (P value determined by Chi-square test was 0.12, 0.59 and 0.92, respectively).

2. As the authors pointed out, a significant proportion (up to 60%) of ACA+ primary Sjogren's syndrome patients developed CREST syndrome in follow-up. It would be helpful to have data on disease/symptom duration (and years of follow-up) on the 2 patient groups. In addition, I would be interested in the authors' view on whether to classify those patients who subsequently develop CREST syndrome as having primary Sjogren's syndrome or CREST syndrome with secondary Sjogren's syndrome or primary Sjogren's syndrome-CREST overlap? This is obviously a controversial area.
According your comments, we calculated the “follow-up period” from point of diagnosis. There was no statistical difference (p=0.16) between ACA+(6.6 ± 5.6) and ACA- (4.5 ± 4.6)

Minor Essential Revisions:
While defining "subsets" of primary Sjogren's syndrome patients is a worthwhile clinical question to address, personally I am not sure how "important" it is from a clinical perspective to know that ACA+ have higher prevalence of Raynaud's phenomenon and lower titre of IgG. The finding of lower focus score and more "fibrotic" changes among ACA+ pSS patients is more interesting, but without information on disease duration for instance make the data harder to interpret, and as mentioned earlier, could this reflect the difference between Ro/La+ versus Ro/La- instead? Therefore, the author should justify why these clinical and laboratory differences between the groups are important

Thank you for your precious comments on the relationship between focus score and serum IgG level in the both groups. Based on no difference with regard to follow-up period, we added next sentences in discussion.

Since there was no statistical difference with regard to follow-up period between ACA+ and ACA- pSS groups in this study, we suggest that the high prevalence and normal IgG in ACA+ pSS is specific for this disease subset.

It would perhaps be more interesting to know whether the ACA+ pSS patients have more (or less) serious complications such as pulmonary hypertension (which can complication CREST patients), lymphoma, and whether the severity of their dryness and fatigue symptoms varies, their long-term prognosis and perhaps response to therapy etc..

Reply
Thank you for your comments on more detail for ACA+ pSS patients. We added information with regard to the prevalence of fatigability, complication and medication in this subgroup as follows;

Page 5, line 8
Two out of 14 ACA+ pSS patients complained fatigability and no hematological disorders such as malignant lymphoma was observed in the medical records. With regard to usage of medications, 2 patients used pilocarpine hydrochloride, other 2 patients used cevimeline hydrochloride hydrate and 1 patient used synthetic saliva spray as oral medication. Regarding ophthalmic drop, 3 patients used artificial tear drop and 1 patient used cyanocobalamin for asthenopia.

Editorial comments:
Please include a 'Competing interests' section between the Conclusions and Authors' contributions. If there are none to declare, please write 'The authors declare that they have no competing interests'.

Reply
We added next sentence after conclusion with regard to competing interests.

The authors declare that they have no competing interest.

Please include an Authors' contributions section before the Acknowledgements and Reference list.

Reply
We include Authors’ contribution as follows;

Authors’ contribution
HN participated in the design of this study and in collecting background data, performed image analysis, statistical analysis and drafted the manuscript. TH provided specimen material for Azan Mallory staining. AK, NI, AO, MT, SY, HI and EK performed critical reading for the manuscript. All authors read and approved the final manuscript.
We strongly encourage you to include an Acknowledgements section.

Reply
According to editorial suggestion, we include next sentences.
We thank Ms. Michiko Karasuyama, clinical engineer of Department of Pathology, Nagasaki University Hospital for her technical assistance. This study is supported in part by Ministry of Health, Labor and Welfare.