Are autoantibodies against a 25-mer synthetic peptide of M3 muscarinic acetylcholine receptor a new diagnostic marker for Sjögren’s syndrome?

We read with great interest the article by Naito and colleagues, who recently proposed the autoantibodies against M3 muscarinic acetylcholine receptor (anti-M3R) as a new diagnostic marker for patients with Sjögren’s syndrome (SS). We have been studying anti-M3R recently and reviewed some theoretical aspects. The results of our work with the same 25-mer synthetic peptide (K-R-T-Y-P-G-E-C-Y-F-I-Q- F-I-S-P-T-T-E-T-G-T-A-I) as used by Naito et al showed a similar prevalence of anti-M3 in patients with SS (table 1). Nevertheless, we did not draw the same conclusions and could not agree with the statement that antibodies against the 25-mer synthetic peptide might be a new diagnostic marker for SS.

We believe that the authors should mention a misleading fact in the article by Bacman et al, which was discussed by Cavill et al and Dawson et al — namely, the sequence of 25-mer synthetic peptide used by Bacman et al was in fact the amino acid sequence from the second extracellular loop of M4 muscarinic acetylcholine receptor. Neither of the two groups were able to detect the activity of anti-M3R with conventional immunological approaches. Furthermore, Gao et al constructed a CHO cell line expressing the human M3R gene and found positive anti-M3R antibodies in 9/11 patients with SS and in none of 11 healthy controls.

The enzyme linked immunosorbent assay (ELISA) used by Naito et al was somewhat similar to our procedure. In our ELISA, Costar medium binding microtitre plates were coated with the same 25-mer peptide in absolute ethanol (10 mg/l), and incubated at 4°C for at least 3 hours. Serum samples were first diluted 1:100 in 1% bovine serum albumin (BSA)/phosphate buffered saline (PBS) (Naito et al 1:50 in 5% BSA/PBS) and incubated for 1 hour at room temperature (Naito et al for 2 hours at 37°C). Optical density values of anti-M3R were not normally distributed in any of our tested groups. Therefore, the cut off value was estimated at the 95th centile of 349 controls. Neither sensitivity nor specificity of the ELISA for SS was improved by binding the synthetic peptide to BSA by a cross linker (N-(glycinebuthyloxy)succinimide ester; Pierce).

In conclusion, it seems that the 25-mer synthetic peptide used in routine immunological techniques does not disclose clinically relevant antibodies, suggesting that a short linear peptide does not depict an adequate epitope for the binding of anti-M3R. Data presented by Gao et al, applying native M3R protein, so far more promising, but they should be verified on a larger group of patients and controls.

P Zigon, B Božič, S Čučnik, B Rozman, M Tomšić, T Kveder
University Medical Centre, Department of Rheumatology, Ljubljana, Slovenia

Correspondence to: Ms T Kveder, Medical University Centre, Department of Rheumatology, Ljubljana, Slovenia

Table 1

<table>
<thead>
<tr>
<th>Abs (μg/OD)</th>
<th>C (n = 349)</th>
<th>SS† (n = 107)</th>
<th>SLE (n = 101)</th>
<th>pSS† (n = 75)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average</td>
<td>117</td>
<td>179</td>
<td>116</td>
<td>159</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>122</td>
<td>266</td>
<td>120</td>
<td>220</td>
</tr>
<tr>
<td>Median</td>
<td>88</td>
<td>92</td>
<td>89</td>
<td>81</td>
</tr>
<tr>
<td>Maximum</td>
<td>1216</td>
<td>1755</td>
<td>872</td>
<td>1162</td>
</tr>
<tr>
<td>Minimum</td>
<td>2</td>
<td>7</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>95th Centile</td>
<td>285</td>
<td>635</td>
<td>359</td>
<td>572</td>
</tr>
<tr>
<td>Number of positive sera</td>
<td>17</td>
<td>16</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td>Percent of positive sera</td>
<td>4.8</td>
<td>15.0</td>
<td>5.9</td>
<td>14.7</td>
</tr>
</tbody>
</table>

*pSS, primary SS; †SS, primary + secondary SS.

Author’s reply

Dr Tanja Kveder et al point out two messages about our paper. Firstly, that our previous results are supported by their further experiments using the same 25-mer synthetic peptides. Secondly, they suggest that our statement that antibodies against the 25-mer synthetic peptide might be a new diagnostic marker for SS is open to criticism.

We agree with Dr Kveder’s comments, in part, because we did not elucidate the function of the anti-25-mer synthetic peptide Abs using M3R transfected cells or HSG cell lines. However, Abs against the second extracellular portion of M3R are detected in a subgroup of patients with SS and the presence of this Ab is significantly associated with anti-SSB Ab. Therefore, we consider that anti-25-mer synthetic peptide Abs might be a new diagnostic marker in a subgroup of patients with SS. Of course, further experiments on the functional analysis using anti-25-mer synthetic peptide and anti-M3R protein Abs would be helpful to clarify the better diagnostic marker in patients with SS.

T Sumida
Department of Internal Medicine, University of Tsukuba, Tsukuba City, Japan, sumida@tm.tusukuba.ac.jp

References


Short course prednisolone for adhesive capsulitis

Adhesive capsulitis is a condition whose pathogenesis remains unclear and for which there is no consensus about the best medical treatment.

Writing recently in the Annals, Buchbinder and her colleagues examined 50 patients (24 receiving active treatment, 26 placebo) from community based rheumatology practices. The trial concluded that a 3-week course of 30 mg prednisolone daily is of
significant short term benefit in adhesive capsulitis, but benefits are not maintained beyond 6 weeks”.

Although the authors were careful with their inclusion criteria, they failed to set a cut off point from the time of onset of pain and stiffness of the shoulder. Their subjects had a mean (SD) duration of symptoms of 25.3 (13.2) weeks. This indicates that some of the participants in this study had had a frozen shoulder for 38.6 weeks or approximately 9 months. The treatment period was limited to 3 weeks, regardless of the duration of symptoms. There were no other interventions. Other reported studies have also included patients with long established adhesive capsulitis. The latter with a mean duration at presentation of 5.5 months before oral corticosteroids were used in a trial.

This study makes an important contribution to the subject, but the authors make the point that future research should evaluate different combinations of treatment and their optimal duration.

Based on my experience, I support this recommendation. I have reported the treatment of 30 patients with idiopathic frozen shoulder (IFS). The mean duration of symptoms before referral was 9 weeks. The treatment consisted of 1–3 intra-articular injections of betamethasone (Celestone Chronodose) followed by oral prednisone 15–20 mg daily, initially for 2 weeks. A home exercise programme was advised. All 30 patients regained full range of movement of the affected shoulder with freedom from pain and without relapse. Future trials should incorporate a treatment group that includes a combination of oral and intra-articular corticosteroids. Double blind trials are problematic given the generally poor outcome for untreated IFS. Patients with frozen shoulder with an onset greater than 16 weeks should be excluded from further trials. IFS is a debilitating condition that is currently perceived as having a poor prognosis. Although it is not life threatening, it has a major impact on quality of life. It is therefore important that rheumatologists establish clear objectives for the management of this condition and educate other medical practitioners of the value of early, active treatment in achieving good outcomes.

W A Douglas
201 Wickham Terrace, Brisbane Qld 4000, Australia; w_douglas@bigpond.net.au

References

Author’s reply
I thank Dr Douglas for his interest and observations about our trial. He has documented his positive anecdotal experience in treating 30 patients with adhesive capsulitis with a combination of intra-articular and oral corticosteroids in a brief letter to the editor. Unfortunately, this has not been published as a full report so no details are provided. It is not clear whether this was an open prospective trial or a retrospective chart review, and, if the latter, whether all patients with adhesive capsulitis were included in the review. Similarly, no numerical data are provided and the time interval between the 1–2 intra-articular steroid injections and the start of oral prednisone was not reported. None the less, his criteria that all patients fully recovered, on average 4.5 weeks from initiation of treatment (although no measure of variance is provided) is noteworthy, lends broad support to the conclusions of our trial, and, we agree, may warrant a formal trial.

We disagree that double blind trials pose a problem trial in studying adhesive capsulitis, as this is the best method for minimising bias in assessment of treatment outcome. Placebo controlled trials are appropriate when there are no known effective treatments, and controlled trials are essential for self limiting conditions such as adhesive capsulitis. While we agree that the study of a painful, disabling condition, most studies have in fact established that it has a good prognosis, with resolution of symptoms in 2–3 years, on average, in the majority of patients.

We also disagree with the suggestion that potential trial participants should be excluded if symptoms have been present for longer than 16 weeks. Although we agree that corticosteroids may be more effective in the earlier phase of adhesive capsulitis, and therefore attempting to limit participation in trials of corticosteroids to those with recent onset of symptoms may appear to have merit, early recruitment has proved universally difficult for trials in this field.

Furthermore, our positive trial, which included participants with an average of 21–25 weeks of symptoms, provides clear evidence that this constraint is not necessary.

R Buchbinder
Department of Clinical Epidemiology, Cabrini Hospital and Monash Department of Epidemiology and Preventive Medicine, Suite 41, Cabrini Medical Centre, 183 Waftettee Rd, Malvern, Victoria, Australia 3144, rachelle.buchbinder@med.monash.edu.au

References

CORRECTION

doi:10.1136/ard.2004.023564corr1


Figure 3 in this article should have been published in colour but mistakenly appeared in black and white. The correct figure has now been inserted in the Online version and subscribers to the journal can see the amended article at http://ard.bmj.com/cgi/content/full/64/8/682

FORTHCOMING EVENTS

Second EULAR Course on Systemic Lupus Erythematosus
4–9 September 2005; San Miniato, Italy
This course for 70 young rheumatologists (age <40) has been designed to provide comprehensive, intensive training on various aspects of this disease. It will deal with the following topics:
• Treatment of SLE, molecular basis of drug action, and pharmacogenetics
• Evaluation of patients with SLE: disease activity, damage, response to treatment
• Renal disease in SLE
• Neurological disease in SLE
• Skin disease in SLE
• Particular problems in SLE: fever, vaccination, pregnancy, haematological manifestations

Contact: Organising secretariat: c/o Clinical and Experimental Rheumatology, Via Santa Maria 31, I-56126 Pisa, Italy.
Tel.: +39-050-40124
Fax: +39-050-502299
Email: scolurse@clinexphrmatol.org

Third International Conference on Neuroendocrine Immune Basis of the Rheumatic Diseases
10–12 September 2005; Genova-Santa Margheritis, Italy
Topic: The clinical translation of the neuroendocrine immune mechanisms of the rheumatic diseases for a better understanding and management of their diagnosis and treatment.

Local organiser: Professor Maurizio Cutolo, Division of Rheumatology, DIMI, University of Genova, Italy
Email: mcutolo@unic.it
Contact: Organising secretariat: Michela Cirelli, EDRA spa, Viale Monza , 133 20125, Milan, Italy
Tel: +39 (0)2 281 72300
Fax: +39 (0)2 281 72399
Email: 3rdnei@edraspa.it

XI Mediterranean Congress of Rheumatology
22–24 September 2005; Heraklion Crete, Greece
The meeting is organised by the Departments of Medicine, Rheumatology, and Clinical Immunology and Allergy, University of Crete.
Contact: Organising Bureau (secretariat and travel office) of the Mediterranean Congress of Rheumatology
Tel: 00 30 210 900600
Fax: 00 30 210 9249836
Email: nikolopoulou@amphitrion.gr

Future EULAR congresses
21–24 June 2006; EULAR 2006, Amsterdam, The Netherlands
13–16 June 2007; EULAR 2007; Barcelona, Spain
11–14 June 2008; EULAR 2008; Paris, France