PostScript

MATTERS ARISING

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,[1] 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[2] and reviewed some theoretical aspects.[3] The results of our work with the same 25-mer synthetic peptide (K-R-T-V-P-P-G-E-C-F-I-Q-F-L-S-E-P-T-I-T-F-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*,[4] which was discussed by Cavill *et al* and Dawson *et al*[5 6]—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.[7]

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*-(γ-maleimidobutyryloxy)succinimide ester; Pierce).

In conclusion, it seems that the 25-mer synthetic peptide used in routine immunological techniques[2 5 6] 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, seem far more promising, but they should be verified on a larger group of patients and controls.

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

Correspondence to: Ms T Kveder, University Medical Centre, Department of Rheumatology, Vodnikova 62, SI-1000 Ljubljana, Slovenia; tanja.kveder@kclj.si

References

1 **Naito Y**, Matsumoto I, Wakamatsu E, Goto D, Sugiyama T, Matsumura R, *et al*. Muscarinic acetylcholine receptor autoantibodies in patients with Sjögren's syndrome. *Ann Rheum Dis* 2005;**64**:510–11.
2 **Žigon P**, Čučnik S. Božič B, Rozman B, Plešivčnik-Novljan M, Tomšič M, *et al*. Antibodies against 25-mer synthetic peptide of M3 muscarinic acetylcholine receptor in patients with Sjögren's syndrome and SLE. In, Conrad K, Sack U, eds. Autoantigens, autoantibodies, autoimmunity, Vol 4.Lengerich:Pabst Science Publishers, 2004:378–9.
3 **Hočevar A**, Tomšič M, Praprotnik S, Hojnik M, Kveder T, Rozman B. Parasympathetic nervous system dysfunction in primary Sjögren's syndrome. *Ann Rheum Dis* 2003;**62**:702–4.
4 **Bacman S**, Berra A, Sterin-Borda L, Borda E. Muscarinic acetylcholine receptor antibodies as a new marker of dry eye Sjögren syndrome. *Invest Ophthalmol Vis Sci* 2001;**42**:321–7.
5 **Cavill D**, Waterman SA, Gordon TP. Failure to detect antibodies to extracellular loop peptides of the muscarinic M3 receptor in primary Sjögren's syndrome. *J Rheumatol* 2002;**29**:1342–4.
6 **Dawson LJ**, Allison HE, Stanbury J, Fitzgerald D, Smith PM. Putative anti-muscarinic antibodies cannot be detected in patients with primary Sjögren's syndrome using conventional immunological approaches. *Rheumatology (Oxford)* 2004;**43**:1488–95.
7 **Gao J**, Cha S, Jonsson R, Opalko J, Peck AB. Detection of anti-type 3 muscarinic acetylcholine receptor autoantibodies in the sera of Sjögren's syndrome patients by use of a transfected cell line assay. *Arthritis Rheum* 2004;**50**:2615–21.

Table 1 Anti-M3R antibodies in patients with Sjögren's syndrome (SS), systemic lupus erythematosus (SLE), and healthy controls (C)

Abs (mOD)	C (n = 349)	SS† (n = 107)	SLE (n = 101)	pSS* (n = 75)
Average	117	179	116	159
Standard deviation	122	266	120	220
Median	88	92	89	81
Maximum	1216	1755	872	1162
Minimum	2	7	2	7
95th Centile	285	635	355	572
Number of positive sera	17	16	6	11
Percent of positive sera	4.8	15.0	5.9	14.7

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

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Author's reply

Dr Tanja Kveder *et al* point out two messages about our paper.[1] Firstly, that our previous results[1] are supported by their further experiments using the same 25-mer synthetic peptides. Secondly, they suggest that our statement that antibodies (Abs) to 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[2] 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.[1] 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 Abs 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; tsumida@md.tsukuba.ac.jp

References

1 **Naito Y**, Matsumoto I, Wakamatsu E, Goto D, Sugiyama T, Matsumura R, *et al*. Muscarinic acetylcholine receptor autoantibodies in patients with Sjögren's syndrome. *Ann Rheum Dis* 2005;**64**:510–11.
2 **Gao J**, Cha S, Jonsson R, Opalko J, Peck AB. Detection of anti-type 3 muscarinic acetylcholine receptor autoantibodies in the sera of Sjögren's patients by use of a transfected cell line assay. *Arthritis Rheum* 2004;**50**:2615–21.

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 participants (24 receiving active treatment, 26 placebo) from community based rheumatology practices.[1] 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.[2 3] 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 was with 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.[4]

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.[5] 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 best practice 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_b_doug@bigpond.net.au

References

1 **Buchbinder R**, Hoving JL, Green S, Hall S, Forbes A, Nash P. Short course prednisolone for adhesive capsulitis (frozen shoulder or stiff painful shoulder); a randomised, double blind, placebo controlled trial. *Ann Rheum Dis* 2004;**63**:1460–9.
2 **Blockey NJ**, Wright JK, Kellgren JH. Oral cortisone therapy in periarthritis of the shoulder. *BMJ* 1954;**1**:1455–7.
3 **Binder A**, Hazelman BL, Parr G, Roberts S. A controlled study of oral prednisolone in frozen shoulder. *Br J Rheumatol* 1986;**25**:288–92.
4 **Douglas WA**. Treatment of idiopathic frozen shoulder with oral and intra-articular corticosteroids. *Aust Musculoskeletal Med* 2004;**9**:7.
5 **Schaeffer B**, Tibone JE, Kerian RK. Frozen shoulder – a long term follow-up. *J Bone Joint Surg Am* 1992;**74**:738–46.

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.[1] 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 claim 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,[2] 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 adhesive capsulitis is 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.[2]

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 trialists in this field.[2]

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 Wattletree Rd, Malvern, Victoria, Australia 3144; rachelle.buchbinder@med.monash.edu.au

References

1 **Douglas WA**. Treatment of idiopathic frozen shoulder with oral and intra-articular corticosteroids. *Aust Musculoskeletal Med* 2004;**9**:7.
2 **Buchbinder R**, Hoving J, Green S, Forbes A, Hall S, Nash P. Short-course prednisolone therapy for the stiff painful shoulder (adhesive capsulitis or frozen shoulder): a randomised placebo-controlled trial. *Ann Rheum Dis* 2004;**63**:1460–9.

CORRECTION

doi: 10.1136/ard.2004.023564corr1

Prevalence and pattern of radiographic hand osteoarthritis and association with pain and disability (the Rotterdam study) (Dahaghin S, Bierma-Zeinstra S M A, Ginai A Z, Pols H A P, Hazes J M W, Koes B W. *Ann Rheum Dis* 2005;**64**:682–7.)

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.bmjjournals.com/cgi/content/full/64/5/682

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Contact: Organising secretariat: c/o Clinical and Experimental Rheumatology, Via Santa Maria 31, I-56126 Pisa, Italy.
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Local organiser: Professor Maurizio Cutolo, Division of Rheumatology, DIMI, University of Genova, Italy
Email: mcutolo@unige.it
Contact: Organising secretariat: Michela Civelli, 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

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The meeting is organised by the Departments of Medicine, Rheumatology, and Clinical Immunology and Allergy, University of Crete.
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Tel: 00 30 210 9006000
Fax: 00 30 210 9249836
Email: nickolopoulou@amphitrion.gr

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