

MATTERS ARISING

Effect of gold treatment on cytokine expression in synovium

A recent paper in this journal described the effect of intramuscular gold treatment on cytokine expression in synovial membranes from patients with rheumatoid arthritis.¹ In that paper, the authors described changes in synovial membrane expression of interleukins (IL) IL-1 α , IL-1 β , IL-6, and tumour necrosis factor α in patients who had received intramuscular gold treatment. However, the authors failed to mention that some of the results had been published in another journal by a similar group of authors.² Neither paper is cross referenced in the other, and it is not clear from these two papers whether the authors are reporting the same set of results in two different papers.

In one paper,² the authors described a significant failure rate with blind needle biopsy of the synovial membrane, which is particularly a problem with studies involving sequential biopsying as was performed in these two studies. Our experience is similar with this technique and we have now changed to performing all synovial biopsying under direct vision through a needle arthroscope under local anaesthesia. However, despite the fact that the two patient groups appear to be similar, if not identical, in these two papers, one paper states that only seven of 18 patients recruited in the study gave three satisfactory sequential synovial biopsy specimens,² while the other paper implies, though does not clearly state, that satisfactory synovial biopsy specimens were obtained in all 10 patients for all time points (0, 2, and 12 weeks).¹ This suggests either that the authors' biopsying technique or success rate has changed dramatically during the period between preparation of the two papers, or that additional patients were obtained to 'make up the numbers'.

Could the authors please confirm whether:

- (1) The patient groups studied in these two papers are, in fact, identical.
- (2) The same results for IL-1 β have been published in two different papers in two different journals.
- (3) All the patients reported in one paper¹ provide adequate synovial biopsy specimens for all three time points and, if so, how was this possible in view of the results and discussion in the other paper from the same group.²

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1 Yanni G, Nabil M, Farahat M R, Poston R N, Panayi G S. Intramuscular gold decreases cytokine expression and macrophage numbers in the rheumatoid synovial membrane. *Ann Rheum Dis* 1994; 53: 315-22.

2 Kirkham B W, Navarro F J, Corkill M M, Panayi G S. In vivo analysis of disease modifying drug therapy activity in rheumatoid arthritis by sequential immunohistological analysis of synovial membrane interleukin 1 β . *J Rheumatol* 1994; 21: 1615-9.

AUTHOR'S REPLY: I would like to respond to Dr Smith's letter as follows:

(1) The study by Kirkham *et al* in the *Journal of Rheumatology* and the paper by Dr Yanni in the *Annals* indeed refer to the same patients.

(2) The paper by Kirkham studied only interleukin-1 β and was the first study reporting the application of immunohistological techniques for cytokines to this kind of material. The paper by Dr Yanni in the *Annals* dealt with four additional cytokines and included additional cellular histological and other markers, particularly related to cells of the monocyte-macrophage series. It was a complete oversight on our part not to have referenced that paper in the *Annals*.

(3) Satisfactory synovial biopsy specimens were obtained in all 10 patients for all the time points (0, 2, and 12 weeks). Some patients refused repeat biopsying or the specimens were technically inadequate; it is for these reasons that not all patients who entered the study were reported upon.

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Cytoplasmic staining, ANA negative status, and ENA testing in rheumatic diseases

Koh and colleagues¹ reported cytoplasmic immunofluorescence staining on HEp-2 cells in 75 of 1173 sera (6.4%) of patients with various rheumatic diseases referred to the Royal National Hospital for Rheumatic Diseases in Bath. Forty two of these 75 sera (56%) were antinuclear antibody (ANA) positive, and 33 ANA negative. Ten of the 75 sera (13.3%) were extractable nuclear antigen (ENA) positive by immunodiffusion (mainly SS-A and Jo-1) and five of these 10 sera were ANA negative. No association of cytoplasmic staining patterns with any specific disease was observed. However, because five of the 10 ENA positive sera were ANA negative, the authors concluded that 'cytoplasmic staining should not be ignored as it may indicate the presence of antibodies to ENA in the absence of nuclear staining'.

We wish to question whether this conclusion is strong enough to advocate positive cytoplasmic staining as an indication for further routine testing for ENA in ANA negative patients with rheumatic diseases. In our laboratory, 5843 sera of patients with various rheumatic diseases were tested for ANA on HEp-2 cells in the past two years. Six hundred sera (10.3%) showed cytoplasmic staining, which is comparable to the 6.4% in Bath. Two hundred and ninety five of those 600 sera with cytoplasmic staining were randomly tested further for ENA by immunodiffusion: 32 (10.8%) were ENA positive (mainly SS-A and SS-B), which is similar to the 13.3% in Bath.

Of the 600 sera with cytoplasmic staining, 179 were ANA positive (29.8%). This percentage is clearly less than that observed in Bath (56%). Theoretically, this discrepancy could be attributable to the study of different patient groups, but the frequency of separate diagnoses did not differ much: systemic lupus erythematosus 12% in Bath *v* 10% in Amsterdam; Sjögren's syndrome 4% *v* 3%; scleroderma 4% *v* 1%; rheumatoid arthritis 12% *v* 17%. These data indicate that the Bath and Amsterdam patient groups are quite similar.

Although our routine cascade testing stops with a negative ANA, 171 ANA negative sera, randomly selected for quality control reasons, were tested for ENA. Of these sera, 152 (89%) did not show any cytoplasmic staining. None of the remaining 19 ANA negative sera which showed a cytoplasmic staining was ENA positive, which is in contrast to the results in Bath, where five of 33 ANA negative sera which showed cytoplasmic staining were ENA positive. Moreover, in our patient group, eight of the 152 ANA negative sera without cytoplasmic staining (5.3%) were ENA positive (eight SS-A, three SS-B, one Sm).

We conclude that it is not useful routinely to follow cytoplasmic staining in HEp-2 cells in the absence of ANA by ENA testing in daily practice for rheumatic patients, because: a) ENA positive sera also occur among ANA negative sera without cytoplasmic staining, and b) the prevalence of positive ENA in ANA negative sera with cytoplasmic staining is rather low: none of 19 in Amsterdam, five of 33 in Bath.

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1 Koh W H, Dunphy J, Whyte J, Dixey J, McHugh N J. Characterisation of anti-cytoplasmic antibodies and their clinical associations. *Ann Rheum Dis*, 1995; 54: 269-73.

AUTHORS' REPLY:

Further to our data, Dr Lems and colleagues provide evidence that a positive anti-ENA may occur in sera that are negative for anti-cytoplasmic and for antinuclear antibodies on routine indirect immunofluorescence of HEp-2 cells. We have not tested a sufficient number of such sera ourselves to assess the relative chance of detecting a positive ENA in the absence of nuclear or cytoplasmic staining. However, we still maintain that cytoplasmic staining may indicate the presence of anti-ENA, especially considering the predominantly cytoplasmic distribution of autoantigens such as Jo-1 (histidyl-tRNA synthetase). Perhaps the important reminder for clinicians is that a negative ANA on immunofluorescence should not preclude further serological testing for autoantibodies when clinically indicated.

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