MATTERS ARISING

Cartilage metabolism—a response

On the basis of measurements of the concentration of aggrecan fragments, cartilage oligomeric matrix protein (COMP), stromelysin, and TIMP in samples of synovial fluid obtained from injured and uninjured knees of subjects with unilateral knee trauma, Dahlberg and colleagues[1] concluded that an abnormality of cartilage metabolism existed in the contralateral knee and suggested that mechanical compensation for the injury, or cytokines or other products released from the injured joint initiated a pathological process in the uninjured knee.

The authors provided reference values for concentrations of the above molecules in a control group of '... 10 healthy athletes without knee symptoms and with no previous knee injury.' However, the median volume of synovial fluid aspirated from the reference group (2·5 ml) was some 3·5 times greater than that from the uninjured knee of subjects with unilateral knee injury, strongly suggesting that the reference group was not 'normal'; unfortunately, results of routine synovial fluid analyses (total leucocyte count and differential) were not provided.

If concentrations of the various markers are normalised for dilution, using the median synovial fluid volume in the uninjured knee of the subjects with unilateral injury (0·7–1·0 ml), the median quantity of aggrecan, for example, present in synovial fluid samples from the reference group was nearly *150% greater* than that in samples from the contralateral knee of the subjects with knee injury, raising doubt about the authors' interpretation of the findings for the contralateral knee.

Other factors may also have confounded the results. Did subjects limit usage of the injured joint as a result of pain? This would have decreased the rate of clearance of protein from the joint space. In contrast, synovial inflammation resulting from the injury would have increased clearance of the 'marker' from the joint space; even low grade synovitis, with a synovial fluid leucocyte count no greater than 1000–2000 cells/mm^3, may increase clearance of protein from the joint space *three- to fourfold.*[2] If the rate of clearance is not controlled for, the authors' conclusion that abnormalities in synovial fluid concentrations of the molecules measured reflect quantitative changes in articular cartilage metabolism may be misleading.[2-4] The possibility that joint structures other than articular cartilage (inflamed synovium, damaged cruciate ligament or meniscus) contributed significantly to the synovial fluid concentrations of the molecules measured also should not be overlooked.

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1 Dahlberg L, Roos H, Saxne T, *et al.* Cartilage metabolism in the injured and uninjured knee of the same patient. *Ann Rheum Dis* 1994; **53:** 823–7.
2 Myers S L, Brandt K D, Eilam O. Even low-grade synovitis significantly accelerates clearance of protein from the canine knee. Implications for measurement of synovial fluid "markers" of osteoarthritis. *Arthritis Rheum* 1995. In press.
3 Wallis W J, Simkin P A, Nelp W B, Foster D M. Intraarticular volume and clearance in human synovial effusions. *Arthritis Rheum* 1985; **28:** 441–9.
4 Levick J R. Synovial fluid: determinants of volume turnover and material concentration. In: Kuettner K, Schleyerbach R, Peyron J G, Hascall V C, eds. *Articular cartilage and osteoarthritis.* New York: Raven Press, 1992; 529–41.

AUTHORS' REPLY:

The intra-articular volume and rate of removal of fluid from the joint have to be considered when interpreting the results of studies of matrix products in synovial fluid.[1 2] We have discussed this previously;[3-5] it is one reason why we *suggested* changes in cartilage metabolism in both knees after unilateral knee injury rather than *concluded* that a difference in cartilage metabolism existed in both knees after unilateral knee injury, as wrongly quoted by Myers and Brandt.

As all the fluid in a joint cannot be aspirated, the total intra-articular fluid volume is not easily established. A possible method for such calculations is to use the concentration difference of a particular molecule between the fluid initially aspirated and in the aspirate obtained after saline lavage. Because of the discomfort for the patients, it is difficult to use this method routinely. However, Geborek *et al*[6] have shown that when aspirated volumes are less than 10 ml there is a considerable discrepancy between aspirated volume and total intra-articular volume. In our healthy athletes, as in the uninjured and injured knees in the chronic phase (more than six months after the injury), the aspirated joint fluid volumes were generally small.[7] Accordingly, the difference in aspirated volume between the reference knees and the injured and uninjured knees may not be significant. Furthermore, the group of athletes had been extensively examined and we considered them healthy.[8 9] We disagree with the approach that Myers and Brandt suggest to normalise for dilution between groups. The proportion of the total synovial fluid that is aspirated in a given subject is much too variable to allow such an approach. Furthermore, the efficiency of aspiration in the contralateral knee joint may be quite different from that of a normal knee. From the experience of the authors, there seems to be a positive correlation between the synovial fluid marker concentration and total amount of the marker in the joint, rather than a 'volume dilution effect'.[3 10]

Unfortunately, we do not have access to the first study referred to by Myers and Brandt, which is still in press; thus it is impossible for us to comment upon these results. In the study they cite by Wallis *et al*,[1] a relationship was demonstrated between the degree of inflammation and the clearance rate in patients with osteoarthritis and rheumatoid arthritis with obvious joint effusion. In our study,[7] the patients did not have rheumatoid arthritis or osteoarthritis, and they did not show any clinical or arthroscopic signs of inflammation. It therefore seems unlikely that a difference in clearance would have contributed significantly to the results. However, if such a difference existed between the injured and uninjured joint, this would only reinforce our suggestion that the contralateral uninjured joint cannot be recommended as the only control joint in synovial fluid studies.

We agree with Myers and Brandt that a change in gait pattern may be the reason for the difference in synovial fluid marker concentration between the injured and uninjured joint.[7] However, the relative importance of changes in joint loading or clearance for the difference in synovial fluid marker concentration between the injured and uninjured joint is not known.

The findings in our study are further supported by a study by Bensen *et al*[11] in which the authors showed evidence for degenerative changes in the articular cartilage of the contralateral, unoperated knee joint in the Pond-Nuki model of osteoarthritis.

We appreciate the interest and comments of Drs Myers and Brandt; however, we feel that their main concern regarding the presence of an abnormality of cartilage metabolism in the uninjured knee is not warranted on the basis of statements in our paper. We suggest, rather than conclude, that cartilage metabolism is altered also in the uninjured knee joint. The underlying mechanism for the observed alterations is not known, which was also stated. However, we believe that the findings support the conclusion that the contralateral knee cannot be recommended as the only control joint in synovial fluid studies of patients with unilateral knee injury.

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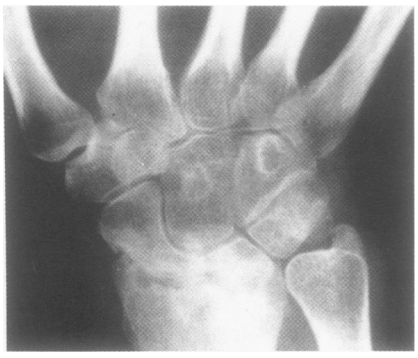
2 Levick J R. Synovial fluid. Determinants of volume turnover and material concentration. In: Kuettner K E, Schleyerbach R, Peyron J, Hascall V C, eds. *Articular cartilage and osteoarthritis.* New York: Raven Press, 1992; 529–41.
3 Lohmander L S, Dahlberg L, Ryd L, Heinegård D. Increased levels of proteoglycan fragments in knee joint fluid after injury. *Arthritis Rheum* 1989; **32:** 1434–42.
4 Dahlberg L, Ryd L, Heinegård D, Lohmander L S. Proteoglycan fragments in joint fluid—influence of arthrosis and inflammation. *Acta Orthop Scand* 1992; **63:** 417–23.
5 Dahlberg L, Fridén T, Roos H, Lark M W, Lohmander L S. A longitudinal study of cartilage matrix metabolism in patients with cruciate ligament injury. Synovial fluid concentrations of aggrecan fragments, stromelysin-1, and tissue inhibitor of metalloproteinase-1. *Br J Rheumatol* 1994; **33:** 1107–11.
6 Geborek P, Saxne T, Heinegård D, Wollheim F A. Measurement of synovial fluid volume using albumin dilution upon intra-articular saline injection. *J Rheumatol* 1988; **15:** 91–4.
7 Dahlberg L, Roos H, Saxne T, *et al.* Cartilage metabolism in the injured and uninjured knee

of the same patient. *Ann Rheum Dis* 1994; **53:** 823–7.
8 Roos H, Dahlberg L, Lohmander L S. Proteoglycan fragments in joint fluid after exercise. *Scand J Med Sci Sports* 1993; **3:** 127–30.
9 Roos H, Dahlberg L, Hoerrner L A, *et al.* Markers of cartilage matrix metabolism in joint fluid and serum after exercise. *Osteoarthritis Cartilage* 1995; **3:** 7–14.
10 Saxne T. Matrix molecules as markers for cartilage involvement in inflammatory joint disease [Dissertation]. Lund: Lund University, 1987.
11 Bensen C V, Dahners L R, Lester G E, Caterson B. Evidence for degenerative changes in articular cartilage of the contralateral 'control' knees in the Pond-Nuki model of osteoarthrosis. *Trans Orthop Res Soc* 1995; **20:** 97.

Autosomal dominant undifferentiated spondyloarthropathy not related to the HLA system

We were interested to read the article by Kidd and colleagues[1] on familial aggregation for undifferentiated spondyloarthropathy associated with HLA-B7. The authors described a single family in whom numerous members had a recurrent seronegative arthropathy or enthesopathy, or both, which fulfilled the European Spondylarthropathy Study Group criteria for spondyloarthropathy[2] in the absence of the HLA-B27 tissue type, coexistent psoriasis, or inflammatory bowel disease. They suggested that 'undifferentiated' spondyloarthropathy can be associated with genetic factors other than HLA-B27. We are in total agreement.

A few years ago, we studied a French family of 83 members distributed over five generations divided into four main branches, among whom 18 adult members had destructive arthropathy and enthesopathic changes.[3 4] In all patients, the disease began between the ages of 18 and 32 years. It affected predominantly the wrists, fingers, shoulders, and peripheral entheses and progressed as an oligoarthritis, with intermittent inflammatory episodes lasting for one to three months. Axial involvement of the cervical and lumbar spine and the sacroiliac joints was also seen, but was not prominent. The sites of involvement seemed to be influenced by mechanical factors. The right wrist was generally the first joint to be affected. Destructive abnormalities, followed by bony proliferation, and intra- or extra-articular bony ankylosis were the main radiological features of this familial arthropathy (figure).



Radiograph of the right wrist of a 29 year old female patient (disease duration six years), showing destruction of the radiocarpal joint.

The transmission of the disease was dominant and autosomal, with 100% penetrance. The clinical and radiological features were strikingly similar in all patients in successive generations and different branches of the genealogical tree, suggesting monogenic transmission. HLA typing of 12 patients and 13 healthy family members was performed. No HLA antigen was linked to the disease. None of the affected subjects had antigens B27, DR4 or DR7. The disease was not transmitted with any particular HLA haplotype.

Tests for rheumatoid factor yielded negative results. There was no history of psoriasis or chronic enteropathy in the members of this family. In none of the 18 patients did the arthropathy fulfil the American Rheumatism Association criteria.[5] A diagnosis of ankylosing spondylitis was also eliminated, because the New York criteria were not fulfilled.[6] This familial arthropathy could belong to the class of undifferentiated spondyloarthropathies proposed by Burns and Calin.[7] The spondyloarthropathies have in common a non-specific inflammation of the entheses, involving both the chondrified and the calcified parts. After a destructive phase, which causes bony erosion, repair takes the form of ossification.[8 9] An inherited abnormality in the collagen matrix of the enthese may predispose to destructive arthropathy and enthesopathic changes. Linkage analysis excluded COL2A1 as the disease causing locus in this family.[10] Further studies are needed to identify the genetic locus responsible for the disease.

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1 Kidd B L, Wilson P J, Evans P R, Cawley M I D. Familial aggregation of undifferentiated spondyloarthropathy associated with HLA-B7. *Ann Rheum Dis* 1995; **54:** 125–7.
2 Dougados M, van der Linden S, Juhlin R, *et al.* The European Spondylarthropathy Study Group preliminary criteria for the classification of spondylarthropathy. *Arthritis Rheum* 1991; **34:** 1218–30.
3 Gaucher A, Weryha G, Dang-Vu V, Moreau P, Péré P. Autosomal dominant spondyloarthropathy. *N Engl J Med* 1989; **320:** 940–1.
4 Gaucher A, Weryha G, Perrier P, *et al.* Autosomal dominant arthropathy in a French family. *Arthritis Rheum* 1991; **34:** 738–43.
5 Arnett F C, Edworthy S M, Bloch D A, *et al.* The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988; **31:** 315–24.
6 Bennett P H, Burch T A. *Population studies of the rheumatic diseases.* Amsterdam: Excerpta Medica, 1968.
7 Burns T A, Calin A. Undifferentiated spondylarthropathies. In: Calin A, ed. *Spondylarthropathies.* Orlando: Grune and Stratton, 1984; 253–64.
8 Ball J. Enthesopathy of rheumatoid and ankylosing spondylitis. *Ann Rheum Dis* 1971; **30:** 213–23.
9 Gaucher A, Péré P, Régent D, Grandhaye P, Aussedat R, Vivard T. Spondylarthropathies ou polyenthésites ossifiantes: arguments scintigraphiques et scanographiques. *Rev Rhum Mal Osteoartic* 1987; **54:** 243–8.
10 Superti-Furga A, Steinmann B, Lee B, *et al.* Autosomal dominant spondylarthropathy: no linkage to the type II collagen gene. *N Engl J Med* 1990; **322:** 552–3.

LETTERS TO THE EDITOR

HLA associations of systemic lupus erythematosus in Chinese from Singapore

A role for genetic factors in systemic lupus erythematosus (SLE) is strongly suggested by the substantial excess recurrence of the disease in monozygotic compared with dizygotic twins.[1] Genes within the major histocompatibility complex (MHC) contribute to SLE, but it has proved difficult to establish the precise locus(i) involved. Studying populations from diverse ethnic backgrounds can help to identify these loci for two reasons: first, there are substantial differences in gene frequencies (for example HLA) between different races; second, different MHC haplotypic combinations of alleles in different races can help to identify the primary relationships with disease, rather than those secondary to linkage disequilibrium. Previous studies in white populations have shown associations with HLA-DR3 and -DR2;[2 3] however, studies from south east Asia, while revealing a strong association with DR2 in Koreans[4] and Chinese from Hong Kong[5] and Malaysia,[6] did not reveal the association with DR3 observed in white subjects.

We studied HLA-DRB1 and -DQB1 alleles in 26 Chinese SLE patients (25 female) attending the National University Hospital in Singapore, and in 77 Chinese controls from the same area. All patients met four or more American Rheumatism Association criteria for SLE. The mean age was 36 years (range 20–64), and mean disease duration 7·1 years (range 1–20). Patients with any known non-Chinese ethnicity (Indians, Malayans, Europeans) were excluded. Renal involvement was present in 57% of the patients, arthritis in 46%, malar rash in 36%, central nervous system involvement in 21%, photosensitivity in 7·1%, and discoid lupus in 3·6%. Antinuclear antibodies were present in 96% of patients and 92% had dsDNA antibodies.

HLA-DRB1 alleles were assigned by polymerase chain reaction (PCR) amplification of genomic DNA probed with sequence specific oligonucleotides.[7] HLA-DQB1 alleles were typed by PCR using sequence specific primer pairs.[8] The statistical significance of differences between the groups was analysed using the χ^2 test.

The table shows the DR and DQ frequencies. There were non-significant increases in DR3, DR8, and DR9, but not in DR2. Furthermore, DQ2 and DQ*0601 were increased in the patients, but this was almost certainly due to linkage with DR3 and DR8, respectively. None of the DR or DQ antigens was associated with particular clinical manifestations of SLE.

Our data are interesting with respect to other published results from Asian populations. HLA-DR9, for instance, was found to be increased in Chinese SLE patients from Hong Kong[5] and Korea, in whom there was