

# Keratan sulphate in rheumatoid arthritis, osteoarthritis, and inflammatory diseases

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## Abstract

Serum concentrations of antigenic keratan sulphate determined by an enzyme linked immunosorbent assay (ELISA) with a monoclonal antibody were studied in patients with rheumatoid arthritis (RA), osteoarthritis, ankylosing spondylitis, other inflammatory diseases, and a large control group of women without arthritis. Mean keratan sulphate concentrations were low in 117 women with RA compared with 227 female control subjects matched for age drawn from a community survey. There were significant correlations between serum keratan sulphate concentrations in patients with RA and serum C reactive protein and the erythrocyte sedimentation rate. Serum keratan sulphate concentrations were also low in 29 men and women with ankylosing spondylitis and 29 patients with arthritis and high concentrations of C reactive protein. In 98 women undergoing an operation for benign breast disease there were decreases in serum keratan sulphate concentrations after the operation which correlated with doses in serum C reactive protein. No differences were found in keratan sulphate concentrations in 137 women with osteoarthritis compared with controls. Within the group with osteoarthritis there were no differences for the various joint groups and there was no obvious correlation with radiographic severity or progression. These findings suggest serum keratan sulphate is unlikely to be useful as a diagnostic marker in osteoarthritis or RA but indicate a role for inflammation in the regulation of cartilage loss.

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The degradation of cartilage is a principal feature of rheumatoid arthritis (RA) and osteoarthritis. The properties of cartilage are dependent on collagen and proteoglycans. Proteoglycans are rich in keratan sulphate chains which appear in synovial fluid and serum samples. The measurement of serum keratan sulphate has been proposed as a marker of cartilage damage in arthritis. Thonar *et al*<sup>1</sup> described an enzyme linked immunosorbent assay (ELISA) using a monoclonal antibody specific for keratan sulphate to determine the amount present in single chains in adult human serum samples. Their initial study reported increased concentrations of keratan sulphate in serum samples from 24 patients with osteoarthritis compared with younger normal adult volunteers and adult control subjects of similar

age in hospital. Subsequent reports from the same unit have confirmed the higher serum levels in 31 men and women with hypertrophic osteoarthritis of the hip<sup>2</sup> and also showed that serum keratan sulphate concentrations increase after chemonucleolysis when there is large scale proteolytic degradation of cartilage.<sup>3</sup> Studies from other centres have produced a less clear cut picture of the value of serum keratan sulphate concentrations.<sup>4 5</sup> Poole *et al*<sup>4</sup> found that low concentrations of keratan sulphate in serum samples were a feature of RA, without a clear explanation of the mechanisms. We therefore examined the value of measuring keratan sulphate as a serum marker of cartilage damage in RA and osteoarthritis and evaluated the relation between concentrations of keratan sulphate in serum and the acute phase response in RA and other inflammatory diseases.

## Patients and methods

### PATIENTS AND CONTROLS

Serum samples were obtained from six groups of subjects. (a) One hundred and seventeen women with RA as defined by the American College of Rheumatology criteria.<sup>6</sup> The patients were 45-70 (mean 56) years old and were attending a specialist rheumatology outpatient clinic. (b) Samples were also obtained from 137 women with osteoarthritis attending an outpatient department; 61 had predominantly osteoarthritis of the knee, 27 had generalised arthritic disease with the hands and knees affected and Heberden's nodes, and 49 had osteoarthritis of either the hand or hip. All were aged 45-88 years (mean 67). In 33 of the patients with osteoarthritis of the knee, paired radiographs were obtained from 11 years previously to assess progression. (c) Twenty nine patients with ankylosing spondylitis attending a specialist rheumatology outpatient clinic who were predominantly male and had a wider age distribution of 22-65 years (mean age 47). (d) Twenty nine patients with Crohn's disease or ulcerative colitis (six men and 23 women; age range 21-58 years) (mean age not available) and an increased serum C reactive protein concentration attending a specialist gastroenterology clinic. (e) Twenty eight women waiting for an operation in whom serum was collected on the day of admission, immediately after the operation, and on days one and three after the operation. The patients were aged 38-81 years (mean 61) and all had an operation for benign breast disease. (f) A large control group of 227 women aged 44-68 years (mean age 60) drawn from an age-sex register of a large local general

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practice in Chingford, East London as part of a survey of rheumatic disease in the community. All the controls were examined clinically and had no evidence of symptomatic joint disease.

#### ENZYME LINKED IMMUNOSORBENT ASSAY FOR KERATAN SULPHATE

Serum keratan sulphate was determined using an ELISA incorporating an inhibition step. The method of Thonar *et al* has been described previously<sup>1</sup> and was used without major modifications. The monoclonal antibody to keratan sulphate (1/20/5-D-4) used is specific for the highly sulphated epitope found at the non-reducing end of long keratan sulphate chains and was developed by Catterson *et al*.<sup>7</sup> This was raised against the proteoglycan core protein which was isolated after chondroitinase ABC digestion of human articular cartilage proteoglycan monomer. The inhibition step was performed at pH 5.3. The coefficients of variation of the assay were 8% for intra-assay variability and 10% for inter-assay variability. The reproducibility was assessed by sending 10 serum samples to two centres: one in Chicago (with the assistance of Dr Eugene Thonar and his colleagues) and the other in Mainz (Dr Klaus Martens and colleagues) who used the same antibody and methodology. A correlation of over 0.9 was achieved with the two centres.

#### OTHER ASSESSMENTS

Patients with RA had the following assessments: duration of morning stiffness recorded in minutes; Ritchie articular index; erythrocyte sedimentation rate; and rheumatoid factor titre (measured using the rheumatoid arthritis particulate assay kit). Serum samples from all patients were also analysed to determine C reactive protein. Women with osteoarthritis of the knee had standing anteroposterior radiographs graded 0-4 using the method of Kellgren and Lawrence by a trained observer blinded to any biochemical or clinical results.<sup>8</sup> In the subgroup of 33 patients with osteoarthritis of the knee the paired radiographs were read independently and blinded to time sequence. Progression was defined by an increase in at least one Kellgren and Lawrence grade; fuller details of the methods are given elsewhere.<sup>8</sup>

#### STATISTICAL ANALYSES

Mean levels were compared by Student's *t* test as keratan sulphate approximated to a normal distribution and log transformation of the data did not alter any of the results. Correlations were made using Spearman's rank correlation coefficient and Wilcoxon's rank sum test was used to assess changes in paired samples. Analysis of covariance was used to examine the effect of age on the results. All the data were entered and analysed using the SPSS PC program.

#### Results

In the 227 female control subjects there was a

weak positive correlation with age ( $r=0.22$ ,  $p<0.001$ ), although there was no effect of years since the menopause, height or weight. The serum keratan sulphate concentrations in the 117 women with RA (mean (SD) 318 (89) ng/ml) was significantly lower ( $p<0.001$ ) than in the control subjects (mean (SD) 389 (93) ng/ml). There were significant inverse correlations between serum keratan sulphate concentrations and serum C reactive protein and the erythrocyte sedimentation rate (table). There was no significant correlation with other indicators of disease activity such as the Ritchie articular index or the duration of morning stiffness.

Mean serum concentrations of keratan sulphate in patients with ankylosing spondylitis and colitis were similar to those seen in RA (fig 1) and there was no significant difference between men and women. Concentrations in patients with these inflammatory diseases were lower than in control subjects, though the control group did not contain any men. Women undergoing elective surgery had an increase in C reactive protein concentrations in serum samples and a decrease in keratan sulphate after the operation. This is illustrated for one patient in fig 2 and the mean changes are shown in fig 3. Statistical analysis of paired results showed highly significant decreases in serum keratan sulphate ( $p<0.001$ ) and increases in C reactive protein ( $p<0.001$ ) between the concentrations before the operation and the third day after the operation.

The mean (SD) serum concentration of keratan sulphate in patients with osteoarthritis was 388 (108) ng/ml. This did not differ from the control group. When subdivided by joint category no major differences between the

Correlations of various clinical and laboratory parameters with concentrations of keratan sulphate in serum samples from 117 patients with rheumatoid arthritis. Spearman's rank correlation coefficients are given

Parameter	Correlation	Probability
C reactive protein	-0.27	0.004
Erythrocyte sedimentation rate	-0.24	0.022
Rheumatoid factor	-0.10	0.301
Articular index	-0.13	0.247
Early morning stiffness	0.10	0.439

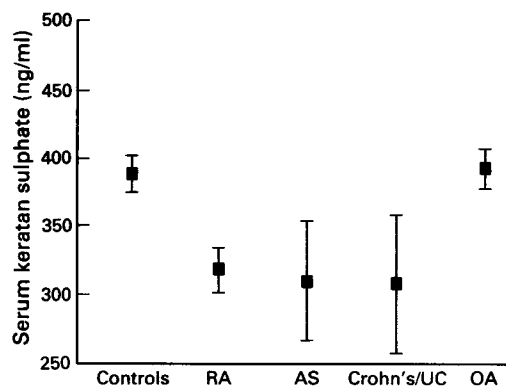


Figure 1 Mean concentrations of keratan sulphate in the various diagnostic groups (95% confidence interval). RA=rheumatoid arthritis; AS=ankylosing spondylitis; Crohn's/UC=Crohn's disease/ulcerative colitis; OA=osteoarthritis.

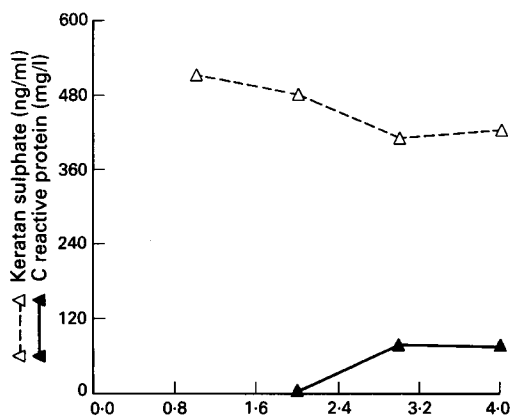


Figure 2 Changes in concentrations of keratan sulphate (y axis) and C reactive protein (x axis) in a patient after an operation.

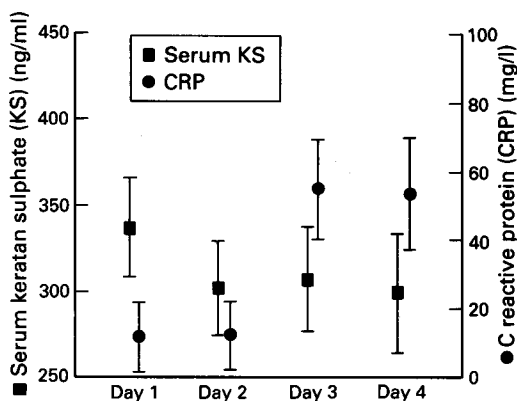


Figure 3 Mean changes in concentrations of keratan sulphate (KS) and C reactive protein (CRP) after an operation.

patients with osteoarthritis were seen: knee 382 (110), generalised osteoarthritic disease 365 (81), and hip and hand 409 (118) ng/ml. Adjusting for the small differences in age between patients with osteoarthritis and control subjects with analysis of covariance did not significantly affect the results: mean 393 ng/ml for controls and 380 ng/ml for patients with osteoarthritis. In the 33 patients with osteoarthritis of the knee followed for 11 years no difference in keratan sulphate concentration was seen in the 15 patients who had progressed at least one Kellgren and Lawrence grade compared with the 18 who had not altered radiologically (mean 406 v 410 ng/ml).

### Discussion

Our data show that compared with control subjects serum keratan sulphate concentrations are low in women with RA and after an operation, but not in patients with osteoarthritis. Concentrations in patients with ankylosing spondylitis and colitis were similar to those in RA, though direct comparison with the control subjects is difficult as no male control subjects were included. These findings suggest that keratan sulphate may be acting as a negative acute phase reactant. This characteristic of keratan sulphate has previously been observed by Poole *et al.*,<sup>4</sup> who found a low mean keratan sulphate concentrations and a negative correla-

tion with serum orosomucoid levels in patients with RA, though there was no correlation with the erythrocyte sedimentation rate. In this study and that of Poole *et al.*<sup>4</sup> correlations with the acute phase indicators have been relatively weak ( $r$  values between 0.2 and 0.3) indicating that the relation only partly explains the changes in serum concentrations of keratan sulphate. The observation by Sweet *et al.*<sup>2</sup> that following the replacement of a single osteoarthritic hip joint serum concentrations of keratan sulphate decreased transiently in all patients but subsequently returned to normal over the following six months could also be explained by the effects of a negative acute phase response. The relation of keratan sulphate with the inflammatory response remains to be elucidated; it is possible, however, that other acute phase reactants might be acting to depress chondrocyte metabolism. Alternatively the clearance of keratan sulphate from the circulation might be increased.

Mehraban *et al.*<sup>5</sup> reported increases in serum keratan sulphate in 43 patients with RA and 58 patients with osteoarthritis compared with 30 patients with fibromyalgia and 27 control subjects from the hospital and laboratory using the same 5-D-4 antibody. No measurements of inflammation were recorded and no details of age and sex of patients were given. Will *et al.*<sup>9</sup> also reported an increase in serum keratan sulphate levels in patients with RA, but Siebel *et al.*<sup>10</sup> reported low keratan sulphate concentrations in 45 patients with RA (compared with 84 control subjects). A number of previous studies have shown increased concentrations of serum keratan sulphate in patients with osteoarthritis compared with control subjects. The study by Seibel *et al.*, however, did not find any significant differences between 45 patients with osteoarthritis and 84 control subjects.<sup>10</sup> A study by Campion *et al.* of 125 patients with osteoarthritis of the knee without control subjects described higher concentrations than the laboratory reference range, with men having significantly higher levels than women.<sup>11</sup> No relation was found with any indicator of disease severity.<sup>11</sup> The discrepancies between our data and those of other workers is not easy to explain. Simple analytical differences are unlikely to explain these conflicting results given that our assay used the same antibody to keratan sulphate as other studies and gave a high correlation with samples from other laboratories. Other workers have found little diurnal variation in keratan sulphate, an apparent long term stability, and no effect from exercise.<sup>3, 12</sup> Although from our data we cannot rule out a difference in keratan sulphate concentrations in men with RA and osteoarthritis, one study has found a sex difference in keratan sulphate concentrations,<sup>11</sup> whereas others have not.<sup>1, 2, 10</sup> and most patients studied were women in all previous reports. An explanation for the disparity in results may lie with the selection of patients and control subjects. We deliberately collected a large representative group of middle aged female control subjects from the general population. Other workers have used either healthy young laboratory staff of mixed sex or

hospital patients. The control subjects in hospital may well have had conditions associated with an acute phase reaction and thus would potentially have a lower serum concentration of keratan sulphate than healthy subjects of the same age. There is also evidence that serum concentrations of keratan sulphate increase with age, at least in children<sup>12</sup> and in our control subjects there was a weak positive correlation with age. If the control subjects are considerably younger than the patients, this could also give misleading results, though in the current study the conclusions were unaltered after adjustment for age. The fact that in patients with osteoarthritis keratan sulphate did not correlate with radiographic severity confirms the study of Campion *et al*<sup>10</sup> and is not surprising given the fact that keratan sulphate reflects normal and diseased cartilage proteoglycans and that synovial joints may only reflect 15% of the body's cartilagenous tissue.<sup>13</sup>

What clinical importance should be given to serum concentrations of keratan sulphate? The evidence from our study suggests that low concentrations follow an acute phase reaction in patients with inflammatory arthritis and in non-arthritic patients. The effect of inflammation and the lack of discrimination in patients with osteoarthritis and correlation with progression implies that low levels of keratan sulphate in the blood do not necessarily reflect changes in cartilage metabolism. This study did not compare serum and local synovial fluid concentrations and there is evidence in osteoarthritis that keratan sulphate concentrations in synovial fluid are higher than those in serum and in some patients might correlate with local joint space loss.<sup>11</sup> We have not identified the mechanism underlying the reduced concentrations of keratan sulphate in serum associated with the acute phase reaction, though the finding implies that serum keratan sulphate has a limited role as a marker of cartilage metabolism in inflammatory arthritis. There is a continuing need to identify an appropriate marker of cartilage destruction that can be used clinically, though the balance of evidence leads us to conclude that keratan sulphate does not fulfil this role. Although a number of other biochemical markers such as hyaluronic acid<sup>14</sup> or urinary collagen crosslinks<sup>15</sup> have been suggested to be useful in monitoring joint damage, it appears

unlikely that any single serum marker will ever be sufficiently sensitive or specific to have a clinical role.<sup>16</sup>

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