

follow changes in marker profiles and the underlying mechanisms which may reflect a restored balance in synthesis and degradation of matrix components.

### Reference

Fenner H. TNF as a target of therapeutic intervention: The immunopharmacological profile of a TNF soluble receptor fusion protein. *Rheumatology in Europe (Suppl 2)* 1994; 23: 17–18.

## Measurement of chondroitin sulphate disaccharides in pathological joint fluids

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A range of extracellular matrix components have been investigated as 'markers' of joint tissue metabolism and many of them have proved to be useful for this purpose. Chondroitin sulphate is the major sulphated glycosaminoglycan present in the extracellular matrix of joint tissues and is an integral component of many proteoglycans. The different molecular forms of proteoglycan (decorin, biglycan, versican, aggrecan etc.) have specific chondroitin sulphation patterns which vary with age and disease.

The purpose of this study was to determine whether chondroitin sulphate disaccharides could discriminate between disease groups and be useful indicators of joint disease severity and progression.

### Methods

Normal synovial fluids were obtained from healthy volunteers with no evidence of joint disease and from patients with osteoarthritis (OA) or rheumatoid arthritis (RA) of the knee. Patients with knee OA were further subdivided according to the presence of calcium pyrophosphate (CPPD) and the presence of nodal OA of the hands (NGOA). In patients with OA or RA, knee inflammation was assessed as active or

inactive using a summated clinical score (Doherty et al. 1988). Synovial fluids were sequentially digested with *streptomyces* hyaluronidase and chondroitin ABC lyase and the resultant unsaturated chondroitin disaccharides were analysed by capillary zone electrophoresis.

### Results

The only sulphated chondroitin disaccharide species detected in synovial fluid were  $\Delta$ di-4S and  $\Delta$ di-6S. The mean  $\pm$  SEM values of  $\Delta$ di-4S and  $\Delta$ di-6S together with the ratios of  $\Delta$ di-6S to  $\Delta$ di-4S for OA and RA patients are shown in Table 1. The total chondroitin sulphate concentration was estimated from the sum of  $\Delta$ di-4S and  $\Delta$ di-6S and was higher in normal fluids than in OA, CPPD and RA fluids. When the distribution of chondroitin sulphate isomers in each group were compared,  $\Delta$ di-6S was the predominant disaccharide, particularly in the normal synovial fluids. The  $\Delta$ di-6S content of all OA groups also appeared to be higher than in RA, but the presence of CPPD or clinical inflammation in the OA groups did not affect the synovial fluid disaccharide composition. Although the concentration of  $\Delta$ di-4S was dis-

Table 1. The chondroitin sulphate disaccharide composition of normal and pathological human synovial fluids, mean  $\pm$  SEM

Synovial Fluid	n	Mean age	$\Delta$ di 6S ng/mg	$\Delta$ di 4S ng/mg	$\Delta$ di6S/ $\Delta$ di 4S
Normal	14	(22–65)	21.43 $\pm$ 2.12	3.93 $\pm$ 0.41	5.49
OA active	10	72.4 (59–88)	9.54 $\pm$ 1.51	3.40 $\pm$ 0.52	2.98
OA inactive	10	71.8 (61–79)	11.30 $\pm$ 2.22	4.30 $\pm$ 0.45	2.46
CPPD active	5	8.7 (59–76)	11.90 $\pm$ 2.18	4.80 $\pm$ 0.79	2.53
CPPD inactive	12	69.5 (66–92)	13.72 $\pm$ 1.77	4.59 $\pm$ 0.72	3.26
NGOA active	5	70.3 (66–73)	9.26 $\pm$ 1.10	6.06 $\pm$ 0.39	1.50
NGOA inactive	8	74.3 (60–87)	10.90 $\pm$ 0.76	6.47 $\pm$ 0.25	1.69
RA active	15	60.1 (28–85)	6.09 $\pm$ 1.02	5.69 $\pm$ 0.63	1.11

tributed over a much narrower range in all synovial fluids, the values were slightly higher for the NGOA and RA groups.

When the  $\Delta$ di-6S: $\Delta$ di-4S ratios were compared, significantly lower values were obtained for all patient groups compared to those measured in normal synovial fluid. However, when the  $\Delta$ di-6S: $\Delta$ di-4S ratios for the three OA patient groups were analysed the lowest values obtained were seen in patients with NGOA and these were comparable to the ratios measured in RA synovial fluids.

### Discussion

The finding that chondroitin sulphate levels of OA and RA joint fluids were significantly lower than those in normal fluids, suggests that the total mass of articular cartilage was decreased. However, the contribution of extra-articular tissues such as the meniscus, cruciate ligament and synovium to the synovial pool should not be underestimated. These tissues also have chondroitin sulphate-containing proteoglycans in their extracellular matrix and the metabolism of these molecules would affect the composition of synovial fluid. Similar mechanisms may also account in part for the lower  $\Delta$ di-6S: $\Delta$ di-4S ratio in OA and RA

fluids compared to those of healthy volunteers.

The only additional clinical feature that was used to differentiate the NGOA group from those patients with knee OA, was the presence of nodal OA of the hands. Even so, this was enough to significantly decrease the  $\Delta$ di-6S: $\Delta$ di-4S ratio seen in these patients. Although the mean synovial fluid values for NGOA patients were very similar to those obtained for RA patients, suggesting that similar biological mechanisms may possibly be involved, it is worth noting that the presence of clinical inflammation in the OA groups did not affect the disaccharide values. This is the first biochemical difference that has been detected in subsets of OA at the same site, and is further justification for considering NGOA as a subset with a possibly different aetiopathogenesis.

### Acknowledgements

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### Reference

Doherty M et al. *Ann Rheum Dis* 1988; 47: 190-7.

## Characterization of proteoglycan populations separated by agarose electrophoresis

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The aim of this study is to develop methods for characterization of small amounts of proteoglycans available from human samples.

### Methods

Proteoglycans (PGs) from 4M GuHCl extracts of normal or arthrotic human knee joint cartilage and from synovial fluids from patients with reactive arthritis or arthrosis were quantitated and purified by precipitation with Alcian Blue (1). The proteoglycans were then separated according to size by agarose electrophoresis (2) and recovered from the gel by capillary blotting.

### Results

*Binding and detection on different membranes.* Conditions of transfer and type of membrane affected both binding and detection of PGs. The PGs were detected on PVDF-P by a reversible Toluidine Blue

staining. Isolated glycosaminoglycans did not bind to PVDF-P. PGs with low protein content did bind to PVDF-P only when blotted at high ionic strength.

*Antigenic epitope identification.* Comparison between Toluidine Blue and immunostaining of the same electrophoretic lane simplifies identification of antigenic epitopes. Recognition of GAG or protein core epitopes with mab 2B6, 5D4, 7D1 and 1C6 often requires deglycosylation.

*Protein core characterization.* The efficacy of deglycosylation with Chondroitinase ABC and Keratanase II of the different stained bands of normal human knee cartilage PGs was evaluated by SDS-PAGE. PGs were either deglycosylated when immobilized on PVDF-P or first released by treatment with guanidine-HCl/propanol and then deglycosylated in solution. Yields of 50-95% depending on type of membrane and staining method were obtained. Largest size proteoglycan population was monodis-