INTRODUCTION:
Biomarkers can have many functions including studying the disease mechanisms, identifying molecular targets for treatment, identifying patients at risk of progression and also monitoring the effects of treatment [1]. Measurement of biomarkers in synovial fluid is often hampered by difficulty in sampling it (due to its high viscosity) and the variable fluid volumes present in the arthritic or injured knee. In the normal knee this is 1-4 cm³, but in the pathological knee can be 10-70 cm³. Thus whereas biomarkers in serum or plasma can be measured reliably per unit volume, this is of no value for synovial fluid. We have measured 2 commonly used biomarkers for joint disease, hyaluronan (HA) and cartilage oligomeric matrix protein (COMP), in a carefully chosen cohort of patients presenting with knee pain in the clinic, in both their synovial fluid and plasma, and normalized levels in the synovial fluid to urea. (The ratio of urea in plasma and synovial fluid has been shown to be constant [2,3]). We are using the same method to measure levels of a newly developed biomarker, that of a ratio of different keratan sulphate (KS) epitopes [4,5] with varying degrees of sulphation, possibly reflecting immature and mature KS chains.

METHODS:
Synovial fluid and plasma was obtained from 30 patients presenting with knee pain in an orthopaedic clinic following their informed consent and with permission of the Local Research Ethics Committee (Shropshire & Staffordshire). Synovial fluid was obtained via a 20mls lavage with saline. Patients underwent an X-ray for Kellgren - Lawrence grading and an arthroscopic examination; this was used to grade them into (i) macroscopically normal (chondral) injury (chondral osteochondral, graded further via Outerbridge classification) or (ii) osteoarthritic changes. 10 samples of synovial fluid and plasma from each group were investigated. Hyaluronan and COMP were measured with commercial ELISA kits (Corgenix Inc and Biovendor Lab Medicine, respectively); HA was measured by a sandwich HA-binding protein assay, with bound bovine cartilage HABP and peroxidase-conjugated HA added, whilst COMP was a sandwich ELISA using an antibody to human COMP. Urea was measured via a colourimetric assay due to urea reacting with o-phthalaldialdehyde and N-1-naphthyl ethylene diamine [6] in a 96 well format (BioAssay Systems). KS epitopes were measured by ELISAs, developed in-house, with the monoclonal antibodies 5D4 and 1B4. 5D4 recognizes linear sequences of N-acetyl galactosamine disaccharides of KS PGs, with both GlcNAc and Gal being sulphated and with a minimal epitope requirement of a keratan sulphate (KS) epitope [4,5] with varying degrees of sulphation, possibly reflecting immature and mature KS chains. Other factors besides the degree of sulphation may, however, influence the level of epitope measured also, for example, the length of the chains and whether the PG it is associated with is bound to the collagen fibril [9].

Non-parametric statistical tests were utilized, eg Mann-Whitney for assessing differences in populations.

RESULTS:
The mean age of the groups of patients was: (i) 22.9 ± 6.1 yrs for macroscopically normal (N), (ii) 30.5 ± 8.8 for those with chondral/osteochondral (CD & OCD) injuries and (iii) 49.3 ± 14.3 for osteoarthritic (OA) changes.

Values for the ratio of KS epitopes for different sulphation patterns (5D4:1B4) ranged from 0.16 to 1.56 in the serum and 0.12 to 0.76 in the synovial fluid. Interestingly the highest ratio in the serum (but almost lowest in the synovial fluid) was from a 16 year old patient with osteoarthritic changes.

DISCUSSION:
It is generally agreed that biomarkers are needed by researchers and clinicians alike to assist in disease diagnosis, and assessment of severity, risk of onset, progression and also efficacy of treatment [7]. Biochemical markers are suggested to be possible alternatives to imaging modalities [8]. To date, however, there are no ideal biomarkers for monitoring joint degeneration and musculoskeletal disease. Levels of potential biomarkers in the plasma/serum can be influenced by many factors including the mechanism and rate by which they are metabolized. Hence in developing any new biomarkers for degenerative joint disease, examining their levels in synovial fluid provides a good starting point, since this is the body fluid closest to the site of degradation of cartilage. Proteoglycans (PGs) not only are one of the most common molecules in cartilage, but also undergo most degradation in arthritic changes in the joint. The sulphation pattern of KS chains on the PGs is known to change during development, growth and degradation of connective tissues. For example, highly sulphated epitopes (identified by 5D4) are expressed in the cornea from an early age in the chick embryo [9]. Other factors besides the degree of sulphation may, however, influence the level of epitope measured also, for example, the length of the chains and whether the PG it is associated with is bound to the collagen fibril [9].

Our preliminary results demonstrate potential for KS epitopes to be measured in both synovial fluid and plasma of patients but more work is required to determine normal ranges, the influence of age and to evaluate their diagnostic powers for subgroups of patients. Normalising levels in synovial fluid to urea provides a useful method for taking account of the variable fluid volumes of the joint with concentrations of COMP increasing in both synovial fluid and plasma with the degree of damage (i.e. lowest in macroscopically normal and highest in OA joints). HA levels, however, were highest in patients with injuries and chondral or osteochondral damage, possibly reflecting a different chronology for releasing these matrix molecules from the tissue, or a difference in their metabolism and half-life. This work supports that of others [3] suggesting that utilising urea to normalize biomarker measurements in synovial fluid will become an important feature in evaluation of biomarkers suitable for monitoring and studying musculoskeletal diseases.

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REFERENCES