Conclusions: We have demonstrated that treatment with CS is capable to reduce PBMC inflammation in rabbits in which chronic arthritis aggravates vascular lesions associated to atherosclerosis. CS evoked a clear improvement in the inflammatory response of the synovial membrane, as well as a decrease in the synovial histopathological lesions. Our results suggest that this drug may have beneficial effects on the treatment of inflammatory synovitis probably acting through the NFkB depending pathway.

A36 DIFFERENTIAL EFFECTS OF TUMOR NECROSIS FACTOR-α AND INTERLEUKIN-1β IN HUMAN CHONDROCYTES. A 2-DE ELECTROPHORESIS APPROACH

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Purpose: Il-1β and TNF-α are cytokines that have always been grouped into the pro-inflammatory cytokine class. In some pathologic scenarios, as in apoptotic environment, chondrocytes respond in a different way depending on what cytokine is present. It is not clear the exact mechanisms by they exercise their different functions in the chondrocyte. The present study, analyzes the differences in protein expression patterns between both cytokines in order to understand the development of rheumatic pathologies.
Methods: Human normal chondrocytes were isolated from cartilage obtained from autopsies without history of joint disease. Chondrocytes were incubated for 48 hours in basal conditions, with Il-1β (5 ng/ml) or TNF-α (10 ng/ml), and cellular protein extracts were obtained. A pool of four patients in each condition, by duplicate, was made and resolved by 2-DE. Protein spots were visualized with Sypro for comparative analyses or with Coomassie stain for protein identification. Qualitative and quantitative analysis were made with PD-QUEST software. After that, protein spots were identified by mass spectrometry using MALDI-TOF/TOF technology. Validation of identified spots was made by real time PCR, western blot and inmunofluorescence.
Results: Different comparative analyses were made in the spots that were matched in every member. We compared protein profiles between basal and Il-1β, basal and TNF-α and between Il-1β and TNF-α conditions. We found a different regulation in the expression ratios of cytoskeleton proteins, modulation of transcription, metabolism, energy production and stress responses between basal and Il-1β as well as, basal and TNF-α stimulated cells. In addition to this, comparing both cytokines, this table shows the qualitative differences in terms of presence (+) or absence (−), between Il-1β and TNF-α conditions. Also, quantitative differences were found in Tryptophanil tRNA synthethase, Translation initiation factor 3, Interferon-induced GTP-binding protein and Argininosuccinate synthase, that were up-regulated more than two fold in the TNF-α treated cells, respect to Il-1β condition. On the other hand, proteins such as Antisense basic fibroblast growth factor or Triose phosphate isomerise, were down-regulated in the TNF-α situation with a ratio of 0.4 (where Il-1β = 1). These differences affect principally to metabolic chondrocyte activity, transcription and protein modifications.

PROT	PAR12	P58	PSB3	PSME2	LDHB	ACSL4	PBEF	MDHM	G3P	ENOA	MATN	ANX5
TNFα/Il-1β	−/+	−/+	+/−	−/+	−/+	−/+	−/+	−/+	−/+	+/−	−/+	−/+

Conclusions: Our studies indicate that the cytokines, Il-1β and TNF-α, have different protein expression patterns in human normal chondrocytes in culture. This study will help us to understand the role that each member plays in pathologic processes that affects chondrocytes.

A37 AGGRECANASE CLEAVAGE IN THE INTERGLOBULAR DOMAIN OF AGGRECAN IS MARKEDLY ELEVATED IN ACUTE ARTHRITIS, OSTEOARTHRITIS AND AFTER KNEE INJURY

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Purpose: Aggrecanase cleavage at the ^{373}Glu-^{374}Ala bond in the interglobular domain of aggrecan, releasing N-terminal ^{374}ARGS fragments, is an early key event in arthritis and joint injuries. Here we quantify the levels of aggrecan and aggrecan ARGS fragments in synovial fluid from knee healthy subjects and from patients with acute arthritis, knee injury and knee osteoarthritis (OA) with the aim of verifying the pathological role of aggrecanase cleavage at this site.
Methods: Knee synovial fluid (SF) from 26 knee healthy individuals (REF) and 269 patients were obtained in a cross sectional study. Patient groups were acute arthritis (AA; n = 48), knee injury (KI; n = 192) and OA (n = 29). The KI group was divided into three subgroups by time after injury: 0−8 weeks (KI-1; n = 64), 9−52 weeks (KI-2; n = 58) and >1 year (KI-3; n = 70). Aggrecan fragments with the N-terminal ^{374}ARGS were assayed by ELISA using a monoclonal antibody (OA-1). Total aggrecan content was analyzed by an Alcian blue precipitation method measuring sulfated glycosaminoglycan (sGAG) and by an ELISA using the monoclonal antibody 1-F21 recognizing a peptide sequence in the keratan sulfate domain. Kruskal-Wallis one way analysis of variance on rank (Dunn's method) was used for multiple group comparisons; $P < 0.05$ was considered significant. Spearman rank order correlation (r_S) was used for comparisons.
Results: Median concentrations of aggrecan ARGS fragments (pmol/ml SF) were elevated in all groups compared to the REF (Table I). The largest elevation (177-fold) was seen in the acute arthritis group, while the knee injury group had a 13-fold elevation and the OA group a 9-fold elevation. Subdivision of the knee injury group showed that elevation of the ARGS concentration in the knee injury group all occurred in the time span of 0−8 weeks after injury (KI-1) which was 130-fold elevated compared to the reference. The release of ARGS fragments into the synovial fluid thereafter did not differ from REF. The SF ARGS concentration correlated positively with the overall aggrecan content measured by Alcian blue precipitation ($r_S = 0.687$) and by the 1-F21 ELISA ($r_S = 0.667$). In contrast to the results of the ARGS immunoassay, when using the Alcian Blue precipitation method only the AA and KI-1 groups differed in aggrecan concentration from the REF group, and no differences between groups were seen when using the 1-F21 assay (Table I).
Conclusions: The observed differences in ARGS concentration in synovial fluid between the diagnostic groups showed that the aggrecanase cleavage at the ^{373}Glu-^{374}Ala bond in the interglobular domain of aggrecan is enhanced in joint pathology, most markedly in acute arthritis and early after knee injury, but also in knee OA. In contrast to methods quantifying human SF total aggrecan (e.g. by sGAG measurements), quantification of ARGS fragments generated by aggrecanases is clearly a more powerful tool in distinguishing healthy from diseased and injured joints. The ARGS ELISA method could be used for monitoring disease progression, predicting clinical outcomes and following the effects of treatment.

Table I: Human SF median concentrations (ranges) of ARGS, sGAG and aggrecan

	ARGS ELISA				Alcian blue precipitation				1-F21 ELISA			
Diagn groups	n	pmol ARGS/ml	Fold diff	P < 0.05	n	µg sGAG/ml	Fold diff	P < 0.05	n	µg aggrecan/ml	Fold diff	P < 0.05
REF	26	0.5 (0.5−3.3)	*1*	*na*	26	61 (5−210)	*1*	*na*	22	132 (20−558)	*1*	*na*
AA	48	88.5 (0.5−961)	177	Y	47	88 (112−528)	1.44	Y	45	261 (12−1912)	1.98	N
1KI	192	6.6 (0.5−946)	13	Y	191	74 (5−728)	1.21	N	189	104 (0.2−1340)	0.79	N
KI-1	64	64.9 (0.5−946)	130	Y	64	121 (39−728)	1.98	Y	62	178 (44−1340)	1.35	N
KI-2	58	0.5 (0.5−266)	1	N	58	63 (16−317)	1.03	N	57	87 (14−759)	0.66	N
KI-3	70	0.5 (0.5−166)	1	N	69	59 (5−245)	0.97	N	70	87 (0.2−791)	0.66	N
OA	29	4.6 (0.5−318)	9	Y	29	58 (8−201)	0.95	N	29	127 (12−482)	0.96	N

REF, knee healthy reference; AA, acute arthritis; KI, knee injury; KI-1, knee injury subgroup (SF aspiration 0−8 weeks after injury); KI-2, knee injury subgroup (SF aspiration 9−52 weeks after injury); KI-3, knee injury subgroup (SF aspiration >52 weeks after injury); OA, knee osteoarthritis.

A38 RELATIONSHIPS BETWEEN CARTILAGE MRI MARKERS, BIOCHEMICAL MARKERS, AND PAIN

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Purpose: The pathogenesis of osteoarthritis (OA) is a complicated line of events in the whole joint, eventually leading to pain and disability. Pain is one of the most critical symptoms of osteoarthritis (OA) and a central clinical end goal. However, the sources of pain are elusive and not well understood – making treatment development challenging. Furthermore, precise quantification of pain is challenging in clinical trials, causing surrogate markers to be desirable. We investigated the association between pain and knee cartilage markers from magnetic resonance imaging (MRI) as well as biochemical markers of bone and cartilage turnover.

Methods: The 21-month longitudinal study included 159 subjects prospectively selected as representative for the general population. From these, only those over 30 years at follow-up were included to ensure a homogeneous population. In total, 123 completed the study, age 58±14, BMI 26±4, 47% female, and 54% with OA (Kellgren and Lawrence, KL ⩾ 1). Radiographs were acquired in a load-bearing semi-flexed position using the SynaFlex (Synarc) and joint space width (JSW) was measured. MRI scans with near-isotropic voxels were acquired from a Turbo 3D T1 sequence from a 0.18T Esaote scanner (40º FA, TR 50 ms, TE 16 ms, scan time 10 minutes, resolution 0.7 mm × 0.7 mm × 0.8 mm) and markers for volume, thickness, smoothness, homogeneity, and curvature were quantified in a fully automatic computer-based framework. Bone resorption was measured by the biochemical marker serum CTX-I (C-terminal telopeptide of collagen type I) and cartilage degradation by urine CTX-II (collagen type II, sample acquired as second morning void). Pain was quantified by a visual assessment scale (VAS). Radiograph and MRI markers were averaged for left and right medial compartments and MRI markers were the sum of tibial and femoral compartments. Cross-sectional biomarker scores and longitudinal changes over 21 months were tested for association with pain and evaluated by correlation (r), t-test of whether scores are higher in the group with pain (p), and odds ratio for high scores relating to pain (OR).

Results: For all markers, the results are listed in Table 1. Cross-sectionally at follow-up, BMI and CTX-II were associated with pain, whereas age, JSW and homogeneity were borderline associated. Longitudinally, the changes from baseline to follow-up were borderline associated with pain at follow-up for homogeneity and CTX-II. For CTX-II and homogeneity this persisted after linear correction for gender and age (GA cor).

Biomarker associations with pain, cross-sectional (C) and longitudinal (L)

	r (C)	p (C)	OR (C)	r (L)	p (L)	OR (L)
Age	0.21	0.053	3.8 *			
Gender	0.02	0.445	1.4			
BMI	0.26*	0.021*	3.3*	−0.10	0.288	1.9
JSW	−0.19	0.062	3.1*	−0.09	0.737	1.2
CTX-I	−0.16	0.028*	2.9	0.09	0.129	2.3
CTX-II	0.29**	0.001**	7.1**	0.05	0.077	3.2*
Volume	−0.13	0.681	1.5	−0.16	0.063	2.3
Thickness	−0.19	0.425	1.3	−0.14	0.866	0.9
Smoothness	−0.13	0.936	0.9	−0.05	1.000	1.1
Homogeneity	0.22*	0.090	2.3	0.20	0.043*	3.3*
Curvature	−0.02	0.549	1.4	0.06	0.973	0.7
CTX-II (GA cor)	0.28*	0.003**	7.1*	0.05	0.077	3.2*
Hom. (GA cor)	0.23*	0.099	2.3	0.20	0.043*	3.3*

Conclusions: The results in this study indicated that pain was associated with the biochemical marker for cartilage breakdown (CTX-II) rather than bone turnover (CTX-I). Furthermore, pain was associated with the cartilage marker related to internal structure (homogeneity) rather than denudation (volume or thickness). Combined, these results may indicate that early stage cartilage breakdown should not be ignored by treatment development targeting pain prevention. Measurement of pain in longitudinal clinical studies is challenging due to poor precision and confounding effects. Therefore, the cross-sectional and longitudinal associations between pain and cartilage homogeneity (that has inter-scan RMS CV 1.0%) merits further research to reveal whether homogeneity may be a suitable surrogate marker for pain.

A39 ALTERED BONE TYPE I COLLAGEN TURNOVER AND MATURATION ARE ASSOCIATED WITH INCREASED CARTILAGE DEGRADATION IN PATIENTS WITH OSTEOGENESIS IMPERFECTA (OI)

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Purpose: Altered subchondral bone remodeling and structure have been suggested to play a role in the development of osteoarthritis (OA). Recent animal studies have shown progressive articular cartilage degradation in a mouse model of osteogenesis imperfecta (OI), a disease caused by mutations in type I collagen (Col I) genes which are associated with abnormal bone matrix. The aim of this study was to investigate whether adult patients with OI used as a human model of bone Col-I defect, are characterized by increased cartilage type II collagen (Col-II) degradation.

Methods: Sixty four patients with mild OI (25 women, 39 men; mean age (SD): 36.2 (11.6) year) participating in a randomized study and 64 sex and age matched healthy controls were included. In patients with OI and controls, we assessed the following bone Col-I and cartilage Col-II parameters using specific biochemical markers: (1) Col I synthesis by serum N-propeptide (PINP), (2) Col-I degradation by urinary fragments of the helical domain of Col-I (Helix-I), (3) Col-I maturation by the urinary ratio of native (α CTX-I) to isomerized (β CTX-I) aspartic acid of Col-I C-telopeptide (α/β CTX-I). Isomerization is a post-translational modification of Col I reflecting the degree of bone matrix maturation which has shown to be associated with altered structure, a high urinary α/β CTX-I ratio being indicative of lower bone matrix maturation and (4) Col-II degradation by urinary C-terminal telopeptide fragments (CTX-II).

Results: As shown on the table, compared to healthy controls, adults with OI had significantly decreased bone Col-I synthesis (lower PINP), increased bone Col-I degradation (higher Helix-I), decreased bone Col-I maturation (higher α/β CTX-I ratio) and a marked increase in cartilage Col-II degradation (high CTX-II). In patients with OI, urinary CTX-II correlated with Helix-I, (r = 0.48, <0.0001) suggesting an association between bone Col I and cartilage Col II degradation.

Conclusions: Cartilage type II collagen degradation is increased in a human model of bone type I collagen defect. These data are in agreement with animal studies suggesting that altered subchondral bone remodeling and structure may be involved in the development of OA.

Subjects	PINP (ng/ml)	Helix-I (μg/mmol Cr)	α/β CTX-I	CTX-II (ng/mmol Cr)
OI (n = 64)	32.3±19.8*	67.7±40.0*	2.04±0.72*	460±576*
Controls (n = 64)	41.8±13.5	42.8±19.4	1.37±0.43	143±62

*p < 0.001 vs controls; results are means±SD.

A40 PROTEOMIC IDENTIFICATION OF SECRETED BIOMARKERS IN AN EXPLANT MODEL OF EARLY OSTEOARTHRITIS

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Purpose: The molecular changes that occur in the early stages of osteoarthritis (OA) are poorly understood. The aim of this study was to use a targeted proteomic approach aimed at identification of secreted biomarkers from equine cartilage explants stimulated with recombinant equine interleukin-1 beta (Il-1β).

Methods: Equine articular cartilage was obtained from weight bearing regions of metatarsophalangeal joints of horses euthanized for purposes other than for research. Full depth cartilage discs were excised with a biopsy punch and washed extensively in PBS and serum free DMEM. Explants were either incubated alone (control), or with Il-1β (10 ng × ml^{-1}) at 37ºC for 5 days. Supernatants were then removed and frozen before trypsin digestion overnight at 37ºC. The reaction was stopped with formic acid and the samples were stored at −80ºC until proteomic analysis by ESI (Electrospray Ionisation) mass spectrometry. Peptides were separated on a C18 pepmap column using a Dionex U3000 chromatography platform and detected in a Bruker HCT PTM discovery ion trap instrument. The four most abundant peptides in each MS scan were selected for fragmentation. Using MASCOT search engine the fragment patterns for each were compared to the mammalian entries in the SwissProt database. The modifications incorporated into the search were: fixed carbamidomethyl cysteine, variable oxidation of methionine and variable deamidation of asparagine and glutamine residues.

Results: Comparative proteomic analysis of the supernatants identified a number of relevant proteins. Tryptic peptides originating from aggrecan core protein, cartilage oligomeric matrix protein (COMP), fibronectin, fibromodulin, thrombospondin-1 (TSP-1) and matrix metalloproteinases MMP-1 and MMP-3 were detected. Several novel and unexpected secreted proteins were also identified including clusterin, cartilage intermediate layer protein-1 (CILP-1) and chondroadherin (CHAD) precursor.

Conclusions: This study has highlighted the presence of several novel biomarkers in the supernatants of cartilage explants stimulated with Il-1β. Many of the identified proteins have putative matrix functions including participation in cell-matrix and matrix-matrix interactions (i.e. fibronectin, TSP-1, COMP, CHAD), matrix turnover (MMP-1, MMP-3) and extracellular