between lnTGF-β levels and age or BMI, and no significant interactions by race or gender were identified. No significant differences were seen for mean serum lnTGF-β levels by rOA status at the knee or the hip in unadjusted or adjusted analyses (Table). There was a trend toward increasing mean lnTGF-β with increasing severity at the knee in the unadjusted analyses (p = 0.080) that was attenuated after adjustment (p = 0.188). There was no association between lnTGF-β levels and unilateral compared to bilateral knee or hip rOA in either unadjusted or adjusted analyses.

Table: Least squared means of lnTGF-β by rOA outcome

Outcome	Unadjusted Mean	95% CI	p value	Adjusted Mean	95% CI	p value
HOA absent	2.80	2.76−2.85	0.713	2.80	2.76−2.85	0.862
HOA present	2.82	2.74−2.90		2.81	2.73−2.89	
KOA absent	2.79	2.74−2.85	0.185	2.80	2.74−2.85	0.352
KOA present	2.84	2.79−2.89		2.83	2.78−2.88	
KOA severity:						
No KOA	2.79	2.74−2.85	n/a	2.79	2.74−2.85	n/a
Mild KOA	2.83	2.77−2.88	0.417*	2.82	2.76−2.88	0.505*
Mod/severe KOA	2.90	2.80−3.00	**0.080***	2.88	2.77−2.99	0.188*

*p for comparison to no OA.

Conclusions: Although serum TGF-β was increased in AA compared to Whites and in women compared to men, there were no significant associations with presence, laterality, or severity of knee or hip rOA, suggesting that serum TGF-β is unlikely to be useful as a stand-alone biomarker in OA studies.

98 COLL2−1, COLL2−1NO2 AND MYELOPEROXIDASE SERUM LEVELS IN EROSIVE AND NON EROSIVE OSTEOARTHRITIS OF THE HANDS

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Purpose: Erosive osteoarthritis of the hand (EHOA) is thought to be an aggressive variant of hand OA characterised by prominent local inflammation and radiographic aspects of bone erosions in interphalangeal joints. Although serum levels of ultrasensitive CRP were found to be higher in patients with EHOA in comparison with those with non-EHOA, biomarkers have not been investigated until now in these patients.
Aims: (1) To determine Coll2−1, Coll2−1NO2 and myeloperoxydase (MPO) levels in serum of patients with EHOA and compare these levels with those of non-EHOA; (2) To investigate relationships between these biomarkers and disease indices of severity and activity in EHOA.
Methods: Coll2−1, Coll2−1NO2 and MPO were measured in 82 patients, 62 with EHOA (57F and 5M, median age 59.5, range 41−74 years) and 20 with non-EHOA (all F, median age 55, range 43−73 years), fulfilling ACR criteria for hand OA. EHOA was defined in the presence of at least one central bone erosion in the interphalangeal joints. Patients were also evaluated for disease duration, number of affected (swollen or deformed) joints, number of clinically active (swollen and painful or tender) (NCAJ), radiographic score (RS), and number of active joints at bone scintigraphy (NSAJ).
Results: Serum levels of Coll2−1NO2 and MPO were higher in patients with EHOA (0.48±0.63 nM and 231.9±119.8 ng/ml, respectively) than in those with non-EHOA (0.23±1.13 nM, p 0.067), although a significance was only reached by MPO (160.15± ng/ml, p < 0.043). No difference was observed between the two groups for serum levels of Coll2−1. No correlations were observed between the substances considered and the other indices of disease activity or severity.
Conclusions: This is the first study demonstrating an increase of some serum biomarkers in EOA. In this context the most interesting biomarker is MPO, although a trend for an elevation was also observed for Coll2−1NO2.

99 A SENSITIVE AGGRECANASE ASSAY FOR *IN-VITRO*/CELLULAR ACTIVITIES AND FOR TESTING BIOLOGICAL SAMPLES

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Purpose: The loss of cartilage matrix in osteoarthritis (OA) is characterized by increased loss of type II collagen and aggrecan by collagenases and aggrecanases, respectively. Depletion of aggrecan is an early and an important event in OA. We have observed ongoing aggrecanase activity in late stage human osteoarthritic cartilage. Inhibition of aggrecanase activity and upstream effectors may offer an attractive therapeutic target for OA. Methods to detect and quantify aggrecanase activity for OA target identification/validation, screening of compounds, and to test activity in biological/clinical samples is gaining great importance. A sensitive aggrecanase ELISA has been developed to detect pM levels of both *in-vitro* as well as cellular aggrecanase activities.
Methods: Either of two custom-made, C-terminal biotinylated, 'aggrecanase cleavage site' (G^{373}-^{374}A) containing aggrecan interglobular domain (IGD) peptides (51/58mer) was used as the substrate. Samples with or without inhibitors were added to the peptide-coated plate and incubated. Cleaved aggrecan was detected by HRP-BC3 (anti-ARGS), aggrecanase activity quantified, and IC50 determined. The assay was also used to measure aggrecanase activity in synovial fluid samples of rats where the animals were oral dosed with drug and knee synovial fluid samples collected after intra-articular injection of aggrecanase. For the cell-based assay format, primary bovine chodrocytes were cultured onto peptide-coated plates followed by Il-1α induction with or without inhibitors. Cellular activity was measured both in cell extracts and in conditioned media to study the efficacy of inhibitors.
Results: The *in-vitro*/cell-based 96-well format aggrecanase activity assay was found to be very selective and sensitive (detection range of 0.01−1 nM aggrecanase). IC50 values of aggrecanase inhibitors tested in the ELISA were comparable to the FRET IC50s. The ELISA used 70−75 fold less enzyme than the FRET assay. IC50 determination was not limited at low nM ranges in the ELISA as compared to the FRET that could not determine IC50s below 30 nM. The assay detected intra-articularly injected aggrecanase activity in the diluted knee synovial fluid samples of rats dosed with drug and determined the inhibitory level reflecting *in-vivo* drug efficacy. In the cell-based assay format, primary bovine chondrocytes produced aggrecanase activity upon Il-1 induction. This activity was detected in the assay enabling a higher-throughput cell based screening of aggrecanase inhibitors as compared to bovine explant cultures.
Conclusions: The sensitive *in-vitro*/cellular aggrecanase activity ELISA uses a longer IGD peptide of aggrecan that better represents the biological substrate as compared to the FRET peptide (10mer). This assay is more sensitive as compared to the FRET and could determine IC50s at low nM levels. The cell-based format of the assay would be useful for chondrocyte-based secondary screening of aggrecanase inhibitors at a higher throughput. This can also be extended to other purposes including identification/validation of OA targets (example: aggrecanase activity in response to knockdown/over expression of potential OA targets in primary human chondrocytes). Thus, the assay has the added advantage of detecting cellular activity that would not be possible with the existing commercial aggrecanase activity assays. The assay has been used to monitor *in-vivo* drug efficacy in animal models. The application of this assay to detect activity in human synovial fluids is currently being evaluated.

100 PREDICTING RADIOGRAPHIC JOINT SPACE NARROWING (JSN) USING BIOMARKERS FOR OSTEOARTHRITIS (OA) CLINICAL TRIALS

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Purpose: To identify biochemical markers that predict OA progression. Such prognostic biomarkers could provide valuable inclusion criteria for clinical efficacy trials to decrease the variability within patient populations as well as to decrease the time required to detect a significant change in the outcome in response to treatment. We studied the relationship between several biochemical markers and radiographic joint space narrowing (JSN) (i.e., change in joint space width (JSW)) in OA patients over 2 years.
Methods: Urine, serum, and plasma samples were collected at 5 visits: 0, 3, 6, 12, and 24 months from healthy (n = 85) and OA human subjects with symptomatic knee OA (n = 75). The diagnosis of OA was based on a combination of pain or stiffness on most days of a month during the past year and the presence of radiographic OA as defined by Kellgren and Lawrence grades (KLG) 0, 1, 2 and 3. Knee radiographs were taken using the Lyon-Schuss protocol at 0 (baseline), 12, and 24 month. Samples were analyzed for the levels of cartilage, bone, and synovium matrix degradation and synthesis markers as well as for markers of