**NOVEL BIOMARKERS FOR OSTEOARTHRITIS ARE LINKED TO PAIN SENSITIZATION, BONE MARROW LESIONS AND CARTILAGE DAMAGE**

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*Abstract:*  
**Purpose:** Osteoarthritis (OA) is a debilitating disease, causing increased pain and reduced function, often over a long period of time. Bone marrow lesions (BMLs) have been shown to associate with pain in large epidemiological studies. There have been few studies to date examining the molecular mediators of pain and tissue damage from OA-derived BML tissue. We recently performed a gene microarray study of OA BML tissue compared with normal non-OA bone. There were 218 genes regulated in BML tissue compared with normal bone. Amongst the most upregulated genes were those implicated in bone/cartilage turnover and pain modulation. In this study we aimed to investigate the protein expression of the products of some of our most highly expressed genes: thrombospondin 4 (TSP4) and matrix metalloproteinase 13 (MMP 13) from participants with OA compared with healthy controls.  
**Methods:** Samples were evaluated from participants in the Pain Perception in Osteoarthritis study of participants with knee OA. We assessed serum and urine samples for biomarker studies. We evaluated three groups: advanced OA, undergoing joint replacement surgery, mild OA, receiving usual care for knee OA including non-steroidal anti-inflammatory agents and/or physical therapies and normal healthy volunteers. Thrombospondin 4 (TSP4) ELISA: TSP4 levels were measured in serum collected at the first visit using the human thrombospondin 4 ELISA kit (DLDEVELOP). Absorbance of each well was read at 450 nm. CTX-II ELISA: for the detection of MMP-13 activity, we measured the levels ofC-terminal telopeptides of type II collagen cleavage products using the Urine Cartilaps (CTX-II) EIA (Immunodiagnostic Systems). Urine samples collected at the first study visit were sampled for type II collagen cleavage products. Matched urine samples were tested for creatinine for normalisation. Absorbance of each well was read at 450 nm. For all ELISA assays, significance across all the groups was compared using Kruskal-Wallis comparisons, with significance considered at p < 0.05. We also performed analysis of variance - ANOVA for group comparisons to assess the influence of structural damage measured by the MRI knee osteoarthritis score (MOAKS) with adjustment for age, BMI and gender as confounders in relation to the biomarkers tested.  
**Results:** Demographics. We evaluated a total of 110 participants with advanced OA (n=72), mild OA (n=32) and healthy volunteers (n=6). (See table)  
For TSP4 serum protein measurements in our cohort, we found that TSP4 levels were undetectable in normal healthy controls. TSP4 levels were raised significantly in the advanced OA group [Mean (SD) (0.228 (0.27)] compared with mild OA groups [Mean (SD) 0.095 (0.148)] and healthy controls (p=0.0046). We also detected a significant correlation between cartilage degradation and TSP4 levels (p=0.014) when adjusting for age, BMI and gender as confounders. We found that urinary CTX-II levels were significantly increased in our advanced OA group [Mean (SD) 447 (269)], compared with the mild OA [Mean (SD) 320 (174)] and healthy control [Mean (SD) 149.8 (81.0)] groups (p=0.0034). There was a trend for increasing breakdown products of type II collagen with worsening OA disease severity. We found significant correlations between CTX-II degradation products and cartilage damage (p=0.047) and Hoffa synovitis (p=0.001) with adjustment of age, BMI and gender as confounders.  
**Conclusions:** In our study, the highest elevations of TSP4 and urinary CTX-II were observed in the advanced OA group. The *de novo* cartilage formation that we observed within BMLs from our microarray study, coupled with the increased transcriptomic expression of MMP-13 observed and the detection of MMP-13 cleavage products in this biomarker study, suggest recapitulation of the embryonic bone development phenotype within OA with detection of MMP-13 cleavage products in the urine potentially from MMP-13 protease activity arising from BMLs and cartilage. We also found that TSP4 was elevated in subjects with advanced OA at the most significant level. TSP4 has been implicated in the inflammatory response to central nervous system injury, presynaptic hypersensitivity and neuropathic pain states. Our new findings raise the possibility of serum TSP4 and urine type II collagen degradation products in combination as pain, BML- and cartilage-specific OA biomarkers.

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| Demographics table | | | |
| Parameter | Advanced OA | Mild OA | Healthy control |
| Age Mean (SD) | 69.1 (7.7) | 62.3 (8.3) | 45 (5.5) |
| BMI Mean (SD) | 32.5 (5.7) | 29.4 (4.4) | 23.3 (3.6) |
| Gender M:F | 17:55 | 7:25 | 0:6 |