incident osteoarthritic at 30 months, respectively, using Poisson regression with GEE in knee-based analyses. We performed additional analyses on a subregion-basis. Given the systemic effects of vitamin K, we also performed person-based analyses examining the relation of vitamin K deficiency with risk for having incident OA in 1 or 2 knees. All analyses were adjusted for age, sex, BMI, BMD and 25(OH)-vitamin D.

Results: Among 1180 participants (62% women, mean age 62±7.9 years, mean BMI 30.1±5.1 kg/m², median vitamin K 1.2 nm), 21% developed incident knee OA over 30 month follow-up and 9.2% were vitamin K deficient at baseline. In the knee-based analyses, compared with those who were not vitamin K deficient, those who were deficient had a 1.43 times higher risk of incident radiographic OA (95% CI 0.98-2.09), and 2.82 times higher risk of incident cartilage abnormality (95% CI 1.26-6.30). Vitamin K deficiency was not statistically significantly associated with incident osteophytes in the knee-based analyses (RR 1.77, 95% CI 0.41-7.55), although this sample was small as most knees had some osteophytes at baseline and were therefore excluded. The risk of incident osteoarthritic on a subregion basis was similar to the knee-based analyses (RR 1.61, 95% CI 0.71-3.66). In the person-based analyses, those who were vitamin K deficient were more likely to develop incident OA in 2 knees versus 0 knees (RR 2.07, 95% CI 1.28-3.36); 2 knees versus 1 knee (RR 2.76, 95% CI 0.99-7.69); and 1 knee versus 0 knees (RR 1.23, 95% CI 1.01-1.50).

Conclusions: In this first longitudinal study of vitamin K in OA, we found that vitamin K deficiency was associated with an increased risk of incident knee OA, extending prior cross-sectional findings, as well as with incident cartilage abnormalities. We could not detect an association with incident osteoarthritic but most knees had osteoarthritic at baseline, limiting sample size for this analysis. Further study of vitamin K is warranted given its potential to be a simple and effective preventive agent.

047
USING OAI CLINICAL DATA TO STUDY THE PROGNOSTIC ROLE OF MENTAL HEALTH
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Purpose: The Osteoarthritis Initiative provides an excellent source of public use data for the study of prognostic factors that may influence pain and functional status of patients with knee osteoarthritis. An extensive literature has examined various prognostic factors but little research has been done to determine if mental health constructs impact future knee pain or function. The goals of this study were to determine whether baseline depression (measured with the CESD), knee related confidence (measured on a Likert scale) and general psychological distress (measured with the SP-12) independently change in pain and functional status outcomes during two years of follow up.

Methods: To define the sampling frame we included only persons who reported baseline pain of 1 or greater on a 0 to 10 scale in at least one knee and had no knee or hip surgery during the two-year follow-up (n=3407). The four outcome variables of interest included performance-based measures of repeated chair standing and the 20 meter walk test, and self report measures of pain and disability (WOMAC) where the worst of the two knees was used as a person-level measurement. Linear mixed effects models were used to assess the association of each mental health variable with the change of each outcome measure over time. Examination of the descriptive data for the entire sample indicated that little change occurred in the outcome measures. As importantly, change was highly dependent on baseline outcome score. For example, persons scoring in the lowest quartile (least pain) on WOMAC Pain at baseline increased their mean score by 0.2 WOMAC points at 1 year while persons in the highest quartile (worst pain) had reductions in their mean 1 year scores of 6.6 WOMAC points. Because change was highly dependent on baseline outcome, we adjusted for the baseline score for all analyses. All variables prognostic of pain or function identified in previously published large sample cohort studies were considered, and covariates significantly associated with the change in outcome at significance level 0.10 were included in the multivariate analyses to determine the independent role of each of the mental health variables on outcome.

Results: Depression was significantly predictive of the change in WOMAC Pain and Disability outcomes, 20 meter walk time and repeated chair stand time. However, given the very small changes overall in the outcomes, the magnitude of change predicted for each year was very small. For example, for WOMAC pain, each point increase in depression at baseline resulted in a 0.02 point increase in WOMAC Pain scores each year. This finding was highly significant (p<0.0008) and very robust (Estimate = 0.02, Standard error = 0.006). Knee confidence was not predictive of change for any outcome. General psychological distress as measured with the SF-12 Mental Health score was predictive of change in 20 meter walk times and WOMAC Pain.

Conclusions: The most consistent psychological predictor of the change in WOMAC and performance outcomes during the two-year follow up was depression. Although depression in particular appears to be a statistically robust predictor of outcome, given that change is very small and highly dependent on baseline status, our results indicate that a considerable degree of depression would be required to have a meaningful effect on future function.

048
URINARY MARKERS, ALPHA CTX AND CTXII, ARE INDICATIVE OF OA SEVERITY AND BONE TURNOVER
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1Duke University Med. Ctr., Durham, NC; 2Nordic BioSci, Diagnosis, Herlev, Denmark

Purpose: Biological markers have the potential to aid in the identification of people in the earliest stage of osteoarthritis (OA), prior to any evidence of radiographic disease, when interventions would be most efficacious.

We have recently shown that bone scintigraphy is a sensitive indicator of symptomatic knee OA and may be a useful marker of early OA events related to bone turnover. Urinary ALPHA CTX, a marker specific for localized high bone turnover, reflecting bone resorption of newly formed type I collagen by osteoclasts, has shown a strong correlation to the number of bone metastases determined by scintigraphy in patients with breast and prostate cancers. A similar pattern of local high bone turnover has been observed by scintigraphy in patients with OA, thus ALPHA CTX was investigated as a potential OA marker. Urinary CTXII, a well studied marker which detects the C-telopeptide of type II collagen, has previously been shown to be associated with radiographic disease and OA progression. We hypothesize that urinary biomarkers related to degradation of types I and II collagen, ALPHA CTX and CTXII, may be indicative of OA severity and correlate to amount of high bone turnover determined by bone scan.

Methods: A total of 159 participants (118 women, 41 men) were included who met ACR criteria for symptomatic OA and had the presence of Kellgren Lawrence grade 1-4 radiographic OA in at least one knee. In addition to KL grade, which represents global OA severity, compartmental OA severity, based on scoring of osteophytes and joint space narrowing (range of 0-3 for each knee) was determined using the standardized OARSI radiographic atlas. Late phase bone scan images, indicative of bone turnover, were obtained 2 hours after administration of 99mTc-MDP. Four views of each knee (anterior, posterior, medial, and lateral) were obtained to precisely localize intensity and site of uptake. Bone scan images were scored semi-quantitatively by two readers at 16 joint sites, each of which was scored on a scale of 0-3, where 0=normal, 1=mild, 2=moderate, and 3=severe. Urine samples were obtained and stored at -90C until analysis. Concentrations of urinary markers, ALPHA CTX and CTXII (Nordic Biosciences) were determined by ELISA and normalized to creatinine concentration. All data were transformed logarithmically to obtain normality. All aspects of this study were approved by the Institutional Review Board.

Results: Urinary ALPHA CTX did not correlate with severity of knee OA based on the static radiographic features (OST and JSN) but did correlate with the dynamic measure of bone turnover based on intensity of bone scintigraphic uptake in the medial knee compartment. Concentrations of urinary CTXII were strongly correlated with knee OA severity based on osteophyte (medial > lateral OST, Table 1), but not degree of joint space narrowing. Urinary CTXII also correlated with the dynamic measure of bone turnover showing a correlation with intensity of bone scintigraphic uptake in the knee (medial > lateral compartment but not the patellofemoral compartment, Table 2).

Discussion: Urinary ALPHA CTX and CTXII are sensitive markers capable of reflecting accelerated bone turnover in patients with knee OA. Both markers correlated with active bone turnover in the knee. In addition, uCTXII correlated with radiographic OST scores. It has been suggested that the main source of uCTXII is remodeling of type II collagen of mineralized tissue including osteophytes and the mineralized interface between cartilage and bone.
bone. This supposition is supported by these data as concentrations of ucTXII were highly correlated to the severity of OA as measured by osteo-
phyte formation, particularly of the medial compartment. The significant 
association of concentrations of collagenous urinary markers, ALPHA CTX 
and CTXII, to knee bone turnover by bone scintigraphy suggests that these 
markers may be useful non-invasive surrogates for active bone turnover in 
knee osteoarthritis.

049 PROTEOMICS APPROACH FOR THE SEARCH OF OA BIOMARKERS IN SERUM

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Purpose: Osteoarthritis (OA) is the most common rheumatic pathology, characterized mainly by cartilage degradation. Despite its high preva-
lence, the diagnosis methods currently available are limited and lacked 
of sensitivity. Therefore, there is a considerable interest pointed in the 
characterization of new specific OA biomarkers in biological fluids. In 
this work we aimed to set up a proteomic method for the large-scale 
identification and quantisation of proteins in serum from OA patients and 
their comparison with healthy donors.

Methods: Serum samples were obtained from OA patients at different 
stages of the disease (50 samples from grade II and 50 from grade IV), 
and 50 control donors. To reduce interindividual variability, samples were 
grouped in pools made from 10 patients. To reduce the dynamic range of 
protein concentrations and be able to identify minority proteins, the top 
grouped in pools made from 10 patients. To reduce interindividual variability, samples were 
used independently to calculate False Discovery Rates.

Results: The immunodepletion and subsequent proteomic analysis of the 
samples led to the identification of around 160 different proteins in the 
samples. Following this procedure, we 
identified with at least 95% confidence were reported. PSPEP program was 
used independently to calculate False Discovery Rates.

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used independently to calculate False Discovery Rates.

Table 1. Serum Proteins altered in OA patients when compared to healthy donors

<table>
<thead>
<tr>
<th>Protein</th>
<th>Early OA (Grade II)</th>
<th>Late OA (Grade IV)</th>
<th>OA (Both stages)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased</td>
<td></td>
<td></td>
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<tr>
<td>Apolipoprotein E</td>
<td></td>
<td></td>
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<tr>
<td>Beta-2-microglobulin</td>
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<tr>
<td>Plasma protease C1 inhibitor</td>
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<tr>
<td>Complement C4-B</td>
<td>Alpha-18-glycoprotein</td>
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<tr>
<td>Heparin cofactor 2</td>
<td>Alpha-2-HS-glycoprotein</td>
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<tr>
<td>Histidine-rich glycoprotein</td>
<td>Apolipoprotein B-100</td>
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<tr>
<td>Thrombomodulin</td>
<td>CD5 antigen-like</td>
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<tr>
<td>Vitamin K-dependent prot. 5</td>
<td>Complement C3</td>
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<tr>
<td>Ig lambda chain C</td>
<td>Ig m Ab chain C</td>
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<tr>
<td>Mannose-binding protein</td>
<td>CN-acetylhexamersoyl-L-alanine amidase</td>
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<tr>
<td>Zinc-alpha-2-glycoprotein</td>
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<tr>
<td>Decreased</td>
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<tr>
<td>Apolipoprotein A-IV</td>
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<tr>
<td>Complement component A-IV</td>
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<tr>
<td>CB beta chain</td>
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<tr>
<td>Glutathione peroxidase</td>
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<tr>
<td>Plasma kallikrein</td>
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<td></td>
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<tr>
<td>Plasma protease C1 inhibitor</td>
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<td></td>
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<tr>
<td>Serum albumin</td>
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<td></td>
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<tr>
<td>Table 2. Bone Scintigraphy</td>
<td>ucTXI and ucTXII</td>
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<tr>
<td>Serum Med</td>
<td>ucTXI</td>
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<tr>
<td>Serum Lat</td>
<td>ucTXI</td>
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<tr>
<td>Serum Med + Lat</td>
<td>ucTXI</td>
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<tr>
<td>Serum PF</td>
<td>ucTXI</td>
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</tr>
<tr>
<td>Serum Total</td>
<td>ucTXI</td>
<td></td>
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</table>

050 THE IDENTIFICATION OF CIRCULATING NATURAL ANTIBODIES AGAINST ENDOGENOUS MEDIATORS IN THE PERIPHERAL BLOOD SERA OF PATIENTS WITH OSTEOARTHRITIS OF THE KNEE: A NEW DIAGNOSTIC FRONTIER

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Purpose: In kOA disease progression and chronic inflammation is prom-
oted by combinations of principle components of angiogenesis, the 
kallikrein-kinin and rennin-angiotensins systems, which play essential 
roles in the maintenance of vascular homeostasis. The humoral immune 
response represents a form of biological amplification of signals that are 
otherwise weak due to very low concentrations of self-antigens, especi-
ally in the early stages of kOA. To investigate the specific presence of NA 
(IgM, IgG, IgA) to EM (BK, ANII, VEGF, bFGF) in the peripheral blood 
sera of patients with kOA and healthy individuals: focusing on novel immune 
disease-associated biomarkers of angiogenesis.

Methods: In order to quantify NA to EM in the sera, we designed, 
synthesized and then used to develop an ELISA, in which antigens coupled 
to matrix was coated on microtiter plates. Serum samples were incubated 
in the plates, after which bound Ig anti-EM was detected with mouse 
anti-human Ig-HRP. Levels NA-EM in a cohort of controls differed by more 
than 100-fold, whereas the fluctuation of EM-NA levels in individuals over 
time was small (coefficient of variation 8%). Following this procedure, we 
examined variations in the levels of NA recognized a panel of self-antigens 
in the sera from healthy individuals and kOA patients. Blood samples were 
obtained from a cohort of 250 patients with symptomatic kOA (age range 
45-79) fulfilling the American College of Rheumatology criteria for OA of 
the knee joint and 250 age- and sex- matched healthy individuals. All 
kOA patients had involvement of the knee joint with typical radiographic 
changes graded KellgrenLawrence classification. Pain was scored on a 
visual analogue scale (VAS) immediately after walking 50 m. All kOA 
patients are with persistent pain longer than 6 months. Parameters for 
function were performed by Lequesne’s functional indexes.

Results: Different classes of NA (IgM, IgG, IgA) to EM (BK, ANII, VEGF, bFGF) were detected with our ELISA protocol in the sera of kOA patients as well 
as in the sera of healthy individuals. At time of inclusion kOA patients 
(100%) had significantly higher serum BK-IgG levels relative to normal