

mainly by the presence of osteophytes and thinning of joint cartilage.

To examine by serum molecular markers the response of bone tissue in early stage knee OA.

Methods: The study group consisted of 147 subjects aged 32-55 (mean 45) years (51 male, 96 female) with chronic knee pain. Half of the female subjects studied were postmenopausal. Altogether 20 and 32 female subjects matched by age and place of residence served as controls.

Bilateral radiographs were graded for tibiofemoral (TF) and patellofemoral (PF) knee OA at baseline and in the 3 year follow-up. At baseline, 55(34%) individuals in the study group had knee joint symptoms but no radiographic findings, 85(53%) were diagnosed with knee OA grade I. Twenty-one subjects (13%) had OA grade II or III, predominantly in the PF region. Radiographic findings of osteophytes and JSN were distributed in the OA group as follows: one-third had only osteophytes, another third had only JSN and the rest had both simultaneously.

At baseline a general marker of bone turnover, serum osteocalcin (S-OC) and propeptide of type I procollagen (S-P1NP) were measured. At baseline and 3 years later validated bone resorption marker serum C-terminal cross-linked telopeptides of type I collagen (S-CTX-I) was assessed by ECLIA (Roche Diagnostics). Statistics: non-parametric methods.

Results: All three markers revealed significant gender differences. S-CTX-I levels were significantly higher in men. Medians for CTx-I for men and women were 0.416 and 0.371 ng/ml ($p=0.033$). S-OC levels were higher in women (medians for men and women 18.6 and 22.6 ng/ml, $p=0.012$). S-P1NP levels trended to be higher in women ($p=0.08$). CTx-I and P1NP levels were significantly higher in female postmenopausal symptomatic subjects compared to female controls.

We were not able to demonstrate significant correlations between knee OA scores and bone markers neither in men nor women. However, in women we observed an association between baseline S-OC and presence of osteophytes localizing in any knee joint compartment ($\rho=0.228$, $p=0.034$). More detailed analysis revealed a correlation between S-OC and TF osteophytes in lateral compartments in premenopausal subjects ($\rho=0.327$, $p=0.032$).

The same was observed with baseline P1NP (but not with baseline CTx-I) that correlated with TF osteophytes in lateral compartments ($\rho=0.269$, $p=0.045$) in women.

In women, S-CTX-I levels were significantly correlated with height and S-OC with age. In men, we did not find an association between knee joint osteophytes and bone markers, but all markers correlated with BMI.

Conclusions: 1. Gender differences have an important effect on the levels of bone markers. Postmenopausal women have significantly increased bone turnover.

2. In case of early knee OA bone turnover is accelerated. Increased synthesis of type I collagen seems to refer to the proliferative bone response expressed by osteophytes.

3. Baseline S-OC and S-P1NP levels seem to have predictive value for definite type of osteophytosis some years later.

4. For interpretation of the results of bone markers one has to consider the important contribution of age, gender and BMI.

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NITROSYLATED N-TELOPEPTIDE OF TYPE III COLLAGEN (IIINYS): A NEW SPECIFIC BIOCHEMICAL MARKER OF OXIDATIVE-INDUCED SYNOVIAL TISSUE METABOLISM IN ARTHRITIS

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Purpose: Nitric oxide (NO) is an important mediator of joint inflammation and destruction in rheumatoid arthritis (RA) and osteoarthritis (OA). Peroxynitrite induced by NO can react with amino acids including tyrosine (Y) to form nitrotyrosine. Increased NO-related species have been found in the joint of patients with OA or RA, especially in synovial tissue. The aim of this study was to develop an immunoassay recognizing nitrosylated N-telopeptide of type III collagen - one of the main constituent of synovial membrane - to monitor oxidative-related joint damage in arthritis.

Methods: We produced a polyclonal antibody raised against a nitrosylated sequence specific of the N-telopeptide of human type III collagen (IIINys). Using IIINys antibody, we performed immunohistochemistry of synovial tissue from 11 patients with knee OA undergoing total joint replacement. We also developed a competitive ELISA to measure IIINys levels in the synovial fluid, serum and urine of healthy controls and patients with OA or RA.

Results: The IIINys antibody did not recognize the non-nitrosylated sequence of type III collagen N-telopeptide, nitrosylated BSA and free nitrotyrosine, indicating high specificity for both nitrosylation and type III collagen sequence. Immunohistochemistry of synovial tissue from patients with knee OA, showed strong IIINys staining in the extracellular matrix, particularly around the synoviocytes and within macrophage-like cells. The ELISA for serum IIINys demonstrated intra and inter-assay CV below 15% and recovery of diluted serum samples ranged from 96.6 to 118.3% (mean: 99%). Detectable levels of IIINys were measured in the synovial fluid, serum and urine of 11 patients with knee OA. Compared to 30 healthy postmenopausal women, serum IIINys levels were increased by an average of 195% ($p<0.0001$) in 30 postmenopausal women with early RA.

Conclusions: We have developed an immunoassay which detects specifically nitrosylated type III collagen N-telopeptide (IIINys). The strong immunoreactivity of IIINys in the synovial tissue of patients with OA and the marked increased serum IIINys levels in patients with RA, suggest that this new biochemical marker should be useful for the investigation of oxidative-induced alterations of synovial tissue in patients with RA or OA.

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WHOLE BLOOD LEAD LEVELS AND PUTATIVE OSTEOARTHRITIS BIOMARKERS IN AFRICAN AMERICAN AND CAUCASIAN WOMEN

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Purpose: The importance of bone turnover is increasingly recognized in osteoarthritis (OA) pathogenesis. Lead (Pb) is a heavy metal that affects many aspects of bone including osteoclasts, osteoblasts, and calcium and vitamin D metabolism. As such, Pb could potentially be involved in OA given the importance of bone in this process. The effect of Pb on cartilage and other joint tissues is not known. No previous studies have evaluated effects

of lead on putative OA biomarkers. We examined whole blood Pb levels and OA biomarkers in African American and white women.

Methods: A total of 339 women in the Johnston County OA Project Metals Exposure Sub-study (mean age 62.5 (9.4) years, 35% African American) had available whole blood, serum, or urine samples for whole blood lead and biomarkers assessments. Whole blood lead was measured by inductively coupled plasma mass spectrometry at the Inorganic Toxicology laboratory, Division of Laboratory Sciences, National Center for Environmental Health, CDC, Atlanta, Georgia. Urine C-telopeptide fragments of type II collagen (CTX-II), cross linked N telopeptide of type I collagen (NTX-I), serum hyaluronic acid (HA) and cartilage oligomeric matrix protein (COMP) were measured by commercially available kits. Radiographic knee OA (rKOA) was defined by Kellgren-Lawrence grades (2-4) with fixed-flexion posterior-anterior knee films. Pearson correlation coefficients were calculated between Log Pb and log of each biomarker. Analysis of covariance models were used to examine associations between blood lead levels and the 4 chosen biomarkers with log transformed biomarkers as outcomes, adjusted for age, race, BMI, and rKOA variables. Effect modification between log Pb and race were examined, with significance defined by p-values < 0.1 for interaction terms.

Results: Median Pb levels were 1.9 ug/dL (0.5-25.4) and were higher in African American women than white women (p < 0.001). In bivariate associations, log Pb was correlated with log CTX-II (r=0.14, p=0.009) and log NTX-I (r=0.22, p<0.0001), but not with log COMP or log HA (r= 0.07 and 0.06, respectively, p>0.20). In adjusted models, log Pb was associated with mean log CTX-II (p = 0.007), NTX-I (p <0.001), and COMP (p = 0.036), but not with mean log HA (p = 0.9). There were no notable race and log Pb interactions.

Conclusions: Mean blood Pb levels are associated with urine CTX-II, urine NTX-I, and serum COMP, but not serum HA in both African American and white women. These data suggest that Pb has an effect not only on bone collagen, but also on type II collagen and non-collagenous matrix proteins. Potential effects of Pb in the pathogenesis of OA, then, are likely to be related to alterations in these factors, but not to effects on synovial inflammation. We are planning further studies to delineate how Pb may affect joint structures and how other factors such as menopause, hormone replacement therapy, and bone density, might influence these associations.

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EFFECT OF OVER-THE-COUNTER DOSES OF NAPROXEN SODIUM ON INHIBITION OF PLATELET CYCLOOXYGENASE-1 IN HEALTHY VOLUNTEERS

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Purpose: Data from a meta-analysis of randomized controlled trials suggest that 1 g of naproxen is not associated with dele-

terious cardiovascular outcomes, perhaps due to its antiplatelet effects. The effect of over-the-counter (OTC) doses of naproxen on platelets is unknown. We compared the antiplatelet effects of OTC doses of naproxen sodium (NAPSO) with a prescription (Rx) dose of NAPSO and low-dose enteric-coated aspirin (EC-ASA).

Methods: Single-center, randomized, open-label, placebo-controlled, 2-period crossover trial in healthy male and female subjects. Subjects were administered 1 of 3 regimens of NAPSO (NAPSO 220 mg twice daily (bid), NAPSO 220 mg 3 times daily (tid), or NAPSO 550 mg bid) or placebo for 7 days. After a washout period of at least 6 days, subjects were crossed over to receive EC-ASA 81 mg once daily for 7 days. The primary endpoint was inhibition of serum thromboxane B2 (TXB2), measured at trough (12 hours after the final dose of NAPSO) and 24 hours after final dose of EC-ASA. Inhibition of serum TXB2 was measured by a commercially available enzyme immunoassay.

Results: A total of 48 subjects were randomized [the intent-to-treat (ITT) population], and 41 (11 NAPSO 220 mg bid, 9 NAPSO 220 mg tid, 11 NAPSO 550 mg bid, 10 placebo) met the criteria for the evaluable population. Baseline characteristics were comparable among the 4 groups. NAPSO demonstrated an aspirin-like effect on platelet aggregation, as measured by inhibition of serum TXB2. The mean (±SD) degree of serum TXB2 inhibition was 97.9% (± 3.20%) for NAPSO 220 mg bid and 99.4% (± 0.77%) for NAPSO 220 mg tid. The inhibitory effects of these OTC doses of NAPSO were similar to the Rx dose of NAPSO 550 mg bid [99.6% (± 0.69%)]. The lower limit of a one-sided 95% CI test for non-inferiority (NAPSO or placebo versus ASA) for each treatment was -1.7% for NAPSO 220 mg bid, -0.2% for NAPSO 220 mg tid, -0.6% for NAPSO 550 mg bid, and -75.8% for placebo (Table 1). All doses of NAPSO were not inferior to EC-ASA 81 mg. Results were confirmed in an analysis of the ITT population.

Conclusions: Over-the-counter doses of NAPSO produced an antiplatelet effect similar to low dose EC-ASA and Rx dose NAPSO, as measured by inhibition of serum TXB2.

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HYALURONAN-LINKED INTER- α -TRYPSIN INHIBITOR HEAVY CHAIN (SHAP), INTERLEUKIN 8, MMP-3 AND HYALURONAN IN HUMAN SYNOVIAL FLUID AND SERUM IN OSTEOARTHRITIS, JOINT INJURY AND INFLAMMATION

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Purpose: Inter- α -trypsin Inhibitor (I α I) occurs in plasma and has protease inhibitory activity. Heavy chains of I α I can bind covalently to hyaluronan (HA) to form a serum derived hyaluronan associated protein, SHAP, releasing bikunin. The transfer of I α I heavy chain to HA is catalyzed by enzyme factors such as TNF α -stimulated gene 6 protein (TSG-6). SHAP potentiates CD44-mediated leukocyte adhesion to hyaluronan substratum, and has putative roles in HA cross-linking and inflammation.

P96 – Table 1. Percent Inhibition of Serum Thromboxane (TXB2) trough/steady state*

	Period 1 Mean (SD)	Period 2 (EC-ASA 81mg) Mean (SD)	Period 1 - Period 2 Mean Difference(SD)	Lower Limit Non-inferiority (5%) One-sided 95% CI
NAPSO 220 mg bid (440 mg) (n=11)	97.9 (3.20)	98.4 (2.54)	-0.5 (2.20)	-1.7
NAPSO220 mg tid (660 mg) (n=9)	99.4 (0.77)	98.0 (2.57)	1.3 (2.46)	-0.2
NAPSO550 mg bid (1100 mg) (n=11)	99.6 (0.69)	97.2 (5.65)	2.3 (5.46)	-0.6
Placebo (n=10)	47.3 (41.03)	99.2 (1.01)	-51.9 (41.18)	-75.8

*Blood drawn 24 hrs after last ASA dose and 12 hrs after last NAPSO dose at steady state