Association between serum and urine biomarkers and lumbar spine individual radiographic features: the Johnston County Osteoarthritis Project


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SUMMARY

Objective: (1) To determine associations between radiographic features of lumbosacral (LS) spine disc space narrowing (DSN) and osteophytes (OST) and joint metabolism biomarkers (serum cartilage oligomeric matrix protein (COMP), hyaluronic acid (HA), collagen neoepitope (C2C), C-propeptide of type II procollagen (CP-II), urine C-terminal cross-linking telopeptide (CTX-II) and N-terminal telopeptide (NTX-I)). (2) To explore interactions with race, gender and low back symptoms.

Design: Cross-sectional analysis of 547 participants enrolled in the Johnston County (JoCo) Osteoarthritis Project from 2003 to 2004. Mean biomarker levels were estimated with linear regression. Proportional and partial-proportional odds models were used to estimate associations. Interactions were tested with likelihood ratio tests at a P-value < 0.10. Biomarkers were natural log (ln) transformed.

Results: Significant differences in mean biomarker levels were found across severity of DSN for lnHA and lnC2C and lnCTX-II across severity of both DSN and OST. Moderate-to-strong associations were found between biomarkers of type II collagen and DSN, whereas associations with OST were weak. An association between lnHA and DSN was seen in women (adjusted odds ratio [aOR] = 1.34 [95% confidence intervals (CI) 1.08, 1.65]) but no association among men (aOR = 0.90 [95% CI 0.63, 1.26]). In Caucasians there was a decreased association with NTX-I and OST (aOR = 0.67 [95% CI 0.49, 0.91]) and no association in African Americans (AAs) (aOR = 1.06 [95% CI 0.76, 1.47]). There was a positive association of lnCOMP with DSN among those with low back symptoms (aOR = 1.82 [95% CI 1.02, 3.27]), but no association in those without low back symptoms (aOR = 0.65 [95% CI 0.35, 1.20]).

Conclusion: Joint metabolism biomarkers suggest biological differences in the pathologic process involved in DSN and OST that may be gender (HA) and ethnicity (NTX-I) specific.

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Introduction

Intervertebral disc degeneration is a common condition with prevalence estimates from community-based studies to be between 50% and 64% (mean age > 65 years). The degenerative process is characterized by loss of water, aggrecan and types I and II collagen, resulting in disc space narrowing (DSN) on plain film radiographs. Vertebral osteophytes (OST) are likely a response to result from cartilage loss, biomechanical stress or trauma to the annular insertion. Vertebral OST prevalence estimates from community-based studies (mean age > 65 years) range between 75% and 94%.

Joint metabolism biomarkers, found in serum and urine, are described according to the tissue(s) in which the markers are abundant and with respect to the molecules they were developed to identify. Type I collagen markers, such as the N-terminal telopeptide (NTX-I), reflect bone turnover or resorption. Type II collagen markers, the C-terminal cross-linked telopeptide (CTX-II)
and the collagenase-generated neoepitope (C2C) are biomarkers of type II collagen degradation, whereas C-propeptide of type II procollagen (CP-II) reflects cartilage synthesis. The non-aggrecan and non-collagenous marker, such as cartilage oligomeric matrix protein (COMP), reflect cartilage degradation but may also be found in bone, ligaments, tendons and vascular smooth muscle. Hyaluronic acid (HA) is a glycosaminoglycan that is indicative of osteoarthritis (OA) and synovial inflammation. These particular biomarkers have been studied in OA of the hip and knee. Only CTX-II has been evaluated for an association with radiographic changes in the lumbar spine.

Our previous work with the Johnston County (JoCo) OA Project has identified that the lumbar spine individual radiographic features (IRFs) of DSN and OST differ substantially from one another in prevalence suggesting a different pathophysiologic process between the two. Furthermore, we found that the associations with demographic, clinical and radiographic knee, hip and hand OA differ between these lumbar spine IRFs. Our primary objective for this analysis was to compare a broad spectrum of serum (COMP, HA, C2C, CP-II) and urine (NTX-I, CTX-II) biomarkers to determine if they would reflect one or both of the OA-related processes represented by lumbar spine DSN and OST. Our second objective was to determine if the associations of biomarkers with lumbar spine radiologic features would differ among race and gender groups and according to the presence of self-reported low back symptoms. We hypothesized that these biomarkers would be independently associated with lumbar spine DSN and OST above and beyond known associations with concomitant appendicular joint radiographic OA.

Methods and materials

Participants

Data for these cross-sectional analyses come from the JoCo OA Project, an ongoing population-based study set in six rural townships of JoCo, North Carolina. The purpose of the JoCo OA Project is to determine the incidence, prevalence and progression of knee, hip, hand, and spine OA. The sampling strategy and recruitment methods used for the JoCo OA Project are described in detail elsewhere. Briefly, baseline enrollment (1991–98) included civilian, non-institutionalized, African American (AA) or Caucasian adult participants ages 45 years and older recruited by probability sampling. The participants for these particular analyses come from 1,015 participants enrolled in the JoCo OA Project during cohort enrichment (T1*, 2003–04), following the first follow-up (T1, 1999–03) of participants initially recruited from 1991 to 98. The T1* cohort enrichment aimed to increase the sample for AAs and younger participants who were lost over the follow-up period from the study initiation. As such, participants at T1* were younger (mean age 59.3 vs 65.8 years) and had a higher proportion of AAs (40% vs 28%) than those at T1; the two groups did not differ according to gender.

Radiographic evaluation

Of the 1,015 participants that underwent clinical examination, 840 had complete radiographic data for DSN and OST. Reasons for not having lumbar spine radiographs included participant refusals ($n = 6$), women of reproductive age ($<50$ years of age) excluded by protocol from having lumbar spine radiographs ($n = 132$), participants exceeding radiographic table weight limit ($n = 23$) or missing ($n = 6$). Lateral lumbar spine films were taken with the participant lying on his/her left side. All lateral lumbar spine radiographs were graded at each lumbar level by a single bone and joint radiologist (JBR) without regard to participants’ biomarker levels. DSN and OST were graded based upon the Burnett Atlas in a semi-quantitative fashion ($0 = \text{none}$, $1 = \text{mild}$, $2 = \text{moderate}$ and $3 = \text{severe}$). The grading for OST was done for each superior and inferior aspect of the anterior face of the lumbar vertebra.

Participants completed weight bearing posterior-anterior knee radiography of both knees ($n = 979$) with a Synaflexer™ (CCBR-Synarc, San Francisco, CA) positioning device, and bilateral hip radiography ($n = 830$) with supine anterior-posterior pelvis radiographs. The presence of a knee prosthesis due to arthroplasty was the primary reason for missing knee radiographic data. Women of reproductive age ($<50$ years, $n = 132$) were not subjected to pelvic radiation; this accounted for the majority of missing hip radiographic data. Posterior-anterior hand radiographs were available for 1,012 participants and were obtained with the beam focused on the third metacarpal-phalangeal (MCP) joint; a total of 30 hand joints bilaterally were graded including: the distal interphalangeal (DIP), proximal interphalangeal (PIP), metacarpal-phalangeal (MCP), carpometacarpal (CMC) and thumb interphalangeal (IP) and MCP joints. All hip, knee and hand radiographs were read for Kellogg–Lawrence (K–L) score by a single bone and joint radiologist (JBR). Inter-rater and intra-rater reliability have been reported previously with a weighted kappa of 0.86 and 0.89 for both the hip and knee. Hip and knee OA, for these analyses, were defined as a K–L score of $2–4$ in at least one extremity. Hand OA was defined, similar to a previous definition, as having at least one hand with a K–L grade of $2–4$ in one DIP joint and in at least two other interphalangeal joints or CMC joints across both hands; participants were excluded on the basis of any MCP with swelling of grade 2 or higher on clinical examination.

Biomarkers

The participants for this biomarkers study came from those participants in T1* who also had serum and urine analyzed for joint metabolism biomarkers as part of the sub-study of the JoCo OA Metal Exposure Sub-study. This sub-study was designed to consist of 1,700 consecutive individuals from T1* and from the original cohort’s first follow-up (T1) who returned for their second follow-up (T2) in 2006–2008. The sample for the current analysis included participants selected from T1* who had complete biomarker analyses performed ($n = 555$). Urinary CTX-II had missing values for 18 participants and urinary NTX-I had a single participant missing value. There were no missing serum biomarker values; however, some participants with biomarker values had missing or unreadable (congenital defect or surgery) lumbar spine radiographs. Therefore, the final sample for these analyses consists of 547 participants that had both complete lumbar spine radiographs and biomarker values from the original T1* enrollment (Fig. 1).

After collection at the clinic visit, blood and sera were separated and stored on ice. Sera were frozen within 8 h of collection at $–20^\circ\text{C}$, then transferred for long-term storage to an $–86^\circ\text{C}$ environment.

Demographic data

At the time of interview, the following data were collected: age; body mass index (BMI) calculated from height measured without shoes and weight measured with a balance beam scale, race (Caucasian/AA); gender; and low back symptoms by asking participants to answer “yes” or “no” to “On most days do you have pain, aching or stiffness in your lower back?”

Definition of biomarkers

Biomarker analyses were conducted at the laboratory of Dr. Virginia Kraus at Duke University Medical Center. Serum HA was measured with the Hyaluronic Acid Test kit (Corgenix, Westminster,
CO). The precision is 3.6–4.7% intra-assay variability and between 5.7% and 7.0% inter-assay variability. Serum COMP was measured using an in-house sandwich enzyme-linked immunosorbent assay (ELISA). The reported precision is 5.8–6.6% intra-assay variability and between 8.7% and 9.7% inter-assay variability. C2C was measured with a competitive ELISA (Ibex, Montreal, CA). The minimum detectable concentration of C2C is 7.3 ng/ml. Intra- and inter-assay precisions are 2.41% and 9.49%, respectively. CP-II was also measured with a competitive ELISA (Ibex, Montreal, CA). The minimum detectable concentration is reported to be 35.1 ng/ml and intra- and inter-assay precisions are 3.68% and 9.08%, respectively. Urinary bone degradation markers, CTX-II (IDS, Boldon, UK) and NTX-I (Osteomark, Princeton, NJ), were measured with a competitive ELISA precisions are 5.86% and 9.08%, respectively. Urinary bone degradation markers, CTX-II (IDS, Boldon, UK) and NTX-I (Osteomark, Princeton, NJ), were measured with a competitive ELISA. The minimum detectable concentration of urinary CTX-II is reported to be 0.20 ng/ml and the intra- and inter-assay precisions are 5.86% and 9.6%, respectively. Urinary bone degradation markers, CTX-II (IDS, Boldon, UK) and NTX-I (Osteomark, Princeton, NJ), were measured with a competitive ELISA. The minimum detectable concentration of urinary CTX-II is reported to be 0.20 ng/ml and the intra- and inter-assay precisions are 5.86% and 9.97%, respectively. Urinary bone degradation markers, CTX-II (IDS, Boldon, UK) and NTX-I (Osteomark, Princeton, NJ), were measured with a competitive ELISA. The minimum detectable concentration of urinary CTX-II is reported to be 0.20 ng/ml and the intra- and inter-assay precisions are 5.86% and 9.97%, respectively. Urinary bone degradation markers, CTX-II (IDS, Boldon, UK) and NTX-I (Osteomark, Princeton, NJ), were measured with a competitive ELISA. The minimum detectable concentration of urinary CTX-II is reported to be 0.20 ng/ml and the intra- and inter-assay precisions are 5.86% and 9.97%, respectively. Urinary bone degradation markers, CTX-II (IDS, Boldon, UK) and NTX-I (Osteomark, Princeton, NJ), were measured with a competitive ELISA. The minimum detectable concentration of urinary CTX-II is reported to be 0.20 ng/ml and the intra- and inter-assay precisions are 5.86% and 9.97%, respectively.

Definition of lumbar spine radiographic features

Since no universal method exists for coding IRF in the lumbar spine, we utilized a summary procedure similar to a previous study. For DSN, the scoring was based upon a 0–15 scale by summing the values of grades 0–3 for each individual lumbar level. For example, a participant would receive a score of 0 if there were no DSN at a single level, a score of 1 if there was mild DSN at a single level, a score of 2 if there was moderate DSN at any one level or any two levels of mild DSN and so forth; a maximum score of 15 is possible representing a grade of 3 (severe DSN) at all five lumbar levels. These scores were collapsed into four mutually exclusive categories based upon the empirical distribution of severity grades in order to reflect greater lumbar spine degenerative severity with increasing scores (0 = scores of 0, 1 = scores of 1 and 2 combined, 2 = scores of 3 and 4 combined and 3 = scores of 5–15 combined).

A similar scoring system was used for superior and inferior (anterior) OST with values that could range from 0 to 30. These scores were also collapsed into four mutually exclusive categories (0 = scores of 0–2, 1 = scores of 3 to 7 combined, 2 = scores from 8 to 12 combined and 3 = scores of 13+).

Statistical analysis

Each biomarker demonstrated a right skewed distribution, so biomarkers were naturally log (ln) transformed. Descriptive statistics were generated for each outcome in the form of means and standard deviations (SDs) for continuous covariates and counts and proportions for categorical covariates. Adjusted mean biomarker levels were estimated using linear regression. Analysis of variance was used to determine differences across categories of severity and adjusted mean levels of each biomarker.

Due to ordinal nature of the outcome variables, proportional odds (polynomial ordinal) logistic regression models were used for all analyses, in which the outcomes met model fit assumptions, defined as having a score test of P > 0.05 and with graphical assessment of trends in the log-odds with incremental coding of the outcomes. The proportional odds model assumes that the odds ratios (ORs) are the same for all possible cut-points dichotomizing the ordinal dependent variable (radiographic OA, for categories see Table I) into high vs low. For violations, an unconstrained partial-proportional odds model has been recommended since it relaxes the strict proportional odds assumption for only those covariates not meeting the assumption. This model, therefore, provides estimates for k – 1 levels of the outcome, where k is equal to the number of categories in the outcome, to best represent the non-proportional effect across levels of the outcome when a single estimate may not. An unconstrained partial-proportional odds model is considered a “nested” version of the proportional odds logistic regression model; therefore, sensitivity analysis was conducted between models where this approach was required. Proportional ORs and their 95% confidence intervals (CI) were estimated from both models.

All models were adjusted for age, race, gender and BMI, as these covariates have been associated with lumbar spine IRF and at least some of our selected biomarkers in previous literature. The confounding effect of the presence of knee, hip or hand OA was explored using a backward stepwise deletion with a change in estimate approach (difference in the model estimate once the covariate was removed). Therefore, knee, hip or hand OA were retained in the final models if there was a change in the estimate of the logit of >5% between each of the biomarkers and either DSN or
OST. To check for independence of associations, each model included the other radiographic feature. Pair-wise interaction terms between each covariate and biomarker were used to assess effect measure modification. Interaction terms were retained in the model if they had a likelihood ratio test P-value of <0.10. All variables were examined for collinearity using variance inflation factors and tolerance values. No outliers were identified in the final models with sensitivity analyses utilizing leverage plots and removal of potential outliers. All analyses were conducted in Stata 10 (Stata Corp, College Station, TX).

**Results**

Basic demographics, clinical characteristics, and presence of knee, hip, or hand OA for each lumbar spine score category of DSN and OST are summarized in Table I. Compared with men, women were slightly more likely to have severe DSN. In contrast, men were more likely to have severe OST. The prevalence of low back symptoms increased monotonically across severity categories of DSN but remained relatively constant across levels of OST severity. The frequency of knee, hip and hand OA increased with increasing severity of both DSN and OST. The mean age of participants was higher in categories of more severe DSN and OST. The mean BMI increased as severity of OST increased whereas remained relatively constant across DSN severity.

**Table I** Demographics and characteristics of the available 547 participants with lumbar spine radiographic data

<table>
<thead>
<tr>
<th>Score category (sample size)</th>
<th>Disc space narrowing</th>
<th>OST</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1–2</td>
<td>3–4</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men, n (%)</td>
<td>90 (43.1)</td>
<td>71 (36.4)</td>
</tr>
<tr>
<td>Women, n (%)</td>
<td>141 (41.7)</td>
<td>123 (34.0)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA, n (%)</td>
<td>99 (47.0)</td>
<td>70 (33.8)</td>
</tr>
<tr>
<td>Caucasian, n (%)</td>
<td>132 (38.4)</td>
<td>124 (36.5)</td>
</tr>
<tr>
<td>BMI, mean (SD)</td>
<td>29.5 (9.2)</td>
<td>29.6 (6.3)</td>
</tr>
<tr>
<td>Age, mean (SD)</td>
<td>58.4 (8.4)</td>
<td>61.6 (9.2)</td>
</tr>
<tr>
<td>Low back symptoms, n (%)</td>
<td>Present</td>
<td>99 (43.0)</td>
</tr>
<tr>
<td>Absent</td>
<td>131 (57.0)</td>
<td>104 (53.6)</td>
</tr>
<tr>
<td>Knee OA, n (%)</td>
<td>Present</td>
<td>49 (21.4)</td>
</tr>
<tr>
<td>Absent</td>
<td>180 (78.6)</td>
<td>131 (70.8)</td>
</tr>
<tr>
<td>Hip OA, n (%)</td>
<td>Present</td>
<td>52 (22.8)</td>
</tr>
<tr>
<td>Absent</td>
<td>176 (77.2)</td>
<td>152 (78.8)</td>
</tr>
<tr>
<td>Hand OA, n (%)</td>
<td>Present</td>
<td>41 (17.8)</td>
</tr>
<tr>
<td>Absent</td>
<td>190 (82.3)</td>
<td>142 (74.0)</td>
</tr>
</tbody>
</table>

Knee and Hip OA defined as a Kellgren–Lawrence (K–L) grade of 2–4. Hand OA was defined as having at least one extremity with a K–L grade of 2–4 in one DIP joint and having at least 2 other interphalangeal joints or CMC joints across both hands.

* There are 18 missing values for CTX-II and 1 missing value for NTX-I.

DSN and OST defined by summing each participant's lumbar spine grade of 0–3 to a score of 0–15 (DSN) 0–30 for superior and inferior vertebral OST.

OST was included as an adjustment variable in all models except with NTX-I. The strongest adjusted associations were between DSN and lnC2C, lnCP-II and lnCTX-II. The relation between lnHA and DSN did not meet model fit assumptions ($P = 0.001$), indicating a violation of the proportional odds assumption, with monotonically increasing ORs from 1.10 (95% CI 0.91, 1.33), 1.38 (95% CI 1.08, 1.78) and 2.18 (95% CI 1.45, 3.29) over categories of DSN. The adjusted associations between biomarkers and OST were weaker when compared to DSN. The estimate for lnCTX-I was on the opposite side of the null for OST when compared to DSN. lnCTX-II demonstrated the strongest association with OST and had a similar strength of association as DSN. However, lnCTX-II did not meet proportional odds model fit assumptions ($P = 0.001$) and the adjusted association was much stronger in the most severe category of OST (OR = 2.82 (95% CI 1.61, 4.94)) compared to categories of lesser severity OR = 1.42 (95% CI 1.02, 1.99) and OR = 1.47 (95% CI 1.10, 1.97), respectively. lnCOMP was not significantly associated with either DSN or OST.

Most biomarkers did not demonstrate significant differences across race, gender or low back symptoms.

The association between lnTX-I and OST was different across race; we observed a significant inverse association of lnTX-I and OST (OR = 0.67; 95% CI 0.49, 0.91) in Caucasians but not in AAs (OR = 1.06; 95% CI 0.76, 1.47). The association between lnTX-I and OST was also different by gender with a significant inverse association among women (OR = 0.65; 95% CI 0.47, 0.89) but not among men (OR = 1.12; 95% CI 0.79, 1.60). The association between lnHA and DSN had a significant moderate association with women (OR = 1.34; 95% CI 1.08, 1.65) while men had no association (OR = 0.90; 95% CI 0.63, 1.26). Among those with low back symptoms (n = 285) there was a strong positive association with lnCOMP and DSN (OR = 1.82; 95% CI 1.02, 3.27) that was not observed in participants without low back symptoms (OR = 0.65; 95% CI 0.35, 1.20).

**Discussion**

Our study examined the relationship between a broad spectrum of biomarkers and lumbar spine IRF within a large bi-racial...
adjusted mean level (95% CI)

OST adjusted mean level (95% CI)

Adjusted geometric mean levels and 95% CI by DSN and OST for each biomarker

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Disc space narrowing</th>
<th>OST</th>
</tr>
</thead>
<tbody>
<tr>
<td>lnCTX-II* (ng/mM Cr)</td>
<td>1.49 (1.15, 1.92)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>lnNTX-I (nMBCE/mMCr)</td>
<td>1.27 (1.01, 1.60)</td>
<td>0.04</td>
</tr>
<tr>
<td>lnCP-II (ng/ml)</td>
<td>1.68 (1.09, 2.61)</td>
<td>0.02</td>
</tr>
<tr>
<td>lnC2C (ng/ml)</td>
<td>1.77 (0.96, 3.24)</td>
<td>0.07</td>
</tr>
<tr>
<td>lnCOMP* (ng/ml)</td>
<td>1.18 (0.76, 1.81)</td>
<td>0.46</td>
</tr>
<tr>
<td>lnHA* (ng/ml)</td>
<td>1.21 (1.01, 1.45)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

C-terminal cross-linked telopeptide (CTX-II) measured in nanomol per millimol, accounting for creatinine clearance. Cross-linked N-telopeptides (NTX-I) measured in nanomol of bone equivalents accounting for creatinine clearance. C-Propeptide (CP-II). Cleavage of type II collagen (C2C). COMP and HA all measured in nanograms per milliliter. All models adjusted for age, race, gender, BMI and the other outcome.

- Adjusted for knee OA.

There are 18 missing values for CTX-II and 1 missing value for NTX-I.

DSN and OST defined by summing each participant’s lumbar spine grade of 0–3 to a score of 0–15 (DSN 0–30 for superior and inferior vertebral OST). All models adjusted for age, BMI, race and gender. lnCTX-II, lnHA, lnC2C, lnCOMP, lnNTX-I, lnCP-II, lnHA* adjusted for knee OA.

Table II

<table>
<thead>
<tr>
<th>Score</th>
<th>Disc space narrowing</th>
<th>OST</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (n = 231)</td>
<td>129.0 (118.2, 140.9)</td>
<td>128.6 (118.2, 140.9)</td>
</tr>
<tr>
<td>1 (n = 194)</td>
<td>129.0 (118.2, 140.9)</td>
<td>128.6 (118.2, 140.9)</td>
</tr>
<tr>
<td>2 (n = 224)</td>
<td>129.0 (118.2, 140.9)</td>
<td>128.6 (118.2, 140.9)</td>
</tr>
</tbody>
</table>

Adjusted geometric mean levels and 95% CI by DSN and OST for each biomarker.

In our community-based cohort. Adding face validity to the results, in several cases, the biomarkers reflected spine pathology in a biologically plausible manner based on their known relative abundance in spinal tissues. For instance, markers of type II collagen, the most abundant protein in the nucleus pulposus, demonstrated moderate-to-strong associations with DSN above and beyond known associations with radiographic knee OA. In contrast, the association between type II collagen biomarkers and OST were weaker. This may be due to the expected lack of type II collagen in a mature (bony) osteophyte since when the osteophyte is first laid down it is a chondrophyte with collagen II, then it undergoes endochondral ossification involving chondral anlage resorption and remodeling into a bony osteophyte that would be visible and gradable on a radiograph. In contrast, markers of less abundant non-collagenous extracellular matrix components (lnHA and lnCOMP) had weak associations with DSN but were responsive to differences in gender and low back symptoms. Furthermore, lnHA demonstrated increased adjusted mean levels with increasing severity of DSN.

Two previous cross-sectional studies have examined the role of CTX-II as a burden of disease biomarker in the spine. Our study findings are generally consistent with both of these studies regarding increasing levels of CTX-II with severity of DSN. However, our results differ from Garnero and colleagues that found no association between CTX-II and vertebral OST. Differences in sample demographics may be one explanation for this difference. We found that the most severe category of OST had a strong association with CTX-II. This category of OST had a higher proportion of men, who had more severe OST. This may help explain the difference between our studies, since their study consisted of women only. Other reasons may include coding scheme or grading scale differences for the lumbar spine radiographic features between studies.

Our findings indicate that associations between some, but not all, lumbar spine IRE and biomarkers differ by subgroups of race and gender. Previous studies have found that women are more likely to have DSN compared to men. In this study, a moderate independent association existed between lnHA and DSN in women. Therefore, lnHA reflects this previously known gender difference in DSN. In addition, our results indicate that there are significant differences in association across subgroups of both gender and race between lnNTX-I and OST. In race and gender subgroups, lnNTX-I reflected a decreased odds of OST. Women are known to have less severe vertebral OST when compared to men and this could be one explanation for the findings here with lnNTX-I. The differences in association we found between lnNTX-I and OST across race...
may also be explained by bone mineral content since racial differences in this process have been established. Although the race and gender differences observed in this study are consistent with differences in bone and spine structure, we were unable to control for the established relationship between decreased bone mineral density and lnNTX-I, which may confound our reported associations.

Previous studies have found a relationship between biomarkers and both hip and knee symptoms, and low back and sciatic symptoms. We explored the relationship between biomarkers and low back symptoms. Previous work by us and others has revealed a modest association between low back symptoms and DSN. Furthermore, the association between DSN and low back symptoms has been found to increase with severity and number of affected lumbar vertebral levels. Due to the multidimensional and complex nature of low back pain, there are challenges to interpreting differences in associations of biomarkers with low back symptoms. However, the association is noteworthy between lnCOMP and DSN among those with and without low back symptoms. Only among participants with low back symptoms, was there a strong direct association between lnCOMP and DSN. Therefore, it is possible that lnCOMP reflects the ongoing degeneration of the intervertebral disc reflected by DSN, resulting in associated symptoms that have been reported with this process.

The confounding effect of concomitant peripheral joint OA may differ based upon specific joint site and the biomarker analyzed. Interestingly, lnNTX-I, associated with DSN, demonstrated no change in estimate when controlling for knee, hip or hand OA. This suggests that these effects may work through a different pathologic process. Hassett and colleagues reported strong associations between both knee and hip OA progression and DSN while no association was found with hand OA. In agreement, none of our analyses were confounded by hand OA, but several required adjustment for knee OA. The association between lnCOMP, lnCTX-II, lnCP-II, lnC2C and lnHA with knee OA has been established in previous literature; therefore, it is not surprising that models with these biomarkers required adjustment due to confounding by the presence of knee OA. Hip OA was not a confounder in any models, and this is consistent with our previous work that showed that hip OA is not independently associated with lumbar spine IRF. This further strengthens the argument that hip OA follows a different etiologic process from that of the lumbar spine. These findings are similar to the previous work by Kraus and colleagues, as they also found differences in the confounding effect of concomitant peripheral joint OA and subsequent adjustment in multivariate models.

A major strength of these analyses is that they were conducted in a well-defined, community-based population in which we examined a broad spectrum of biomarkers across a balanced representation of AAs and Caucasians and both men and women. Moreover, we were able to analyze lumbosacral (LS) spine associations with biomarkers in the context of concomitant knee, hip, and hand radiographic OA. The primary limitation of this study was its cross-sectional design; thus we could not address the temporal relationship between the onset of serum biomarker abnormalities and onset of OA; this can only be assessed using longitudinal analyses. Our study population does not reflect the general population in so far as our cohort contained a greater proportion of AAs and had a younger mean age. We adjusted for hip, knee, and hand OA to take into account their potential contribution to biomarker levels; however, we did not control for such things as medication use, liver function, and kidney function, which may affect serum levels of biomarkers. All our variables had attendant measurement error. Assuming independent, non-differential errors, our estimates of associations would be an understimation of the true associations. Despite these limitations, we conclude that our findings indicate that these bone and joint biomarkers reflect differences in the process of degeneration between DSN and OST. Furthermore, some of these biomarkers may show differences in lumbar spine degeneration in both race and gender subgroups and the presence of low back symptoms. The ability of biomarkers to independently reflect metabolic processes in the lumbar spine provides the impetus for future studies to determine if biomarkers can also predict degenerative changes in the lumbar spine.

Contributions

All authors of this work have made substantial contributions to the conception and design, acquisition of data, analysis, interpretation, drafting of manuscript and final approval of submission.

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Conflict of interest

All authors disclose they have no financial or personal relationships with other people or organizations that could potentially and inappropriately bias their work and conclusions.

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