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ORIGINAL RESEARCH

Association of Biomarkers with Individual and Multiple Body Sites of Pain: The Johnston County Osteoarthritis Project

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Introduction: Biochemical biomarkers may provide insight into musculoskeletal pain reported at individual or multiple body sites. The purpose of this study was to determine if biomarkers or pressure-pain threshold (PPT) were associated with individual or multiple sites of pain.

Methods: This cross-sectional analysis included 689 community-based participants. Self-reported symptoms (ie, pain, aching, or stiffness) were ascertained about the neck, upper back/thoracic, low back, shoulders, elbows, wrist, hands, hips, knees, ankles, and feet. Measured analytes included CXCL-6, RANTES, HA, IL-6, BDNF, OPG and NPY. A standard dolorimeter measured PPT. Logistic regression was used determine the association between biomarkers and PPT with individual and summed sites of pain.

Results: Increased IL-6 and HA were associated with knee pain (OR=1.30, 95% CI 1.03, 1.64) and (OR=1.32, 95% CI 1.01, 1.73) respectively; HA was also associated with elbow/wrist/hand pain (OR=1.60, 95% CI 1.22, 2.09). Those with increased NPY levels were less likely to have shoulder pain (OR=0.56, 95% CI 0.33, 0.93). Biomarkers HA (OR=1.50, 95% CI 1.07, 2.10), OPG (OR=1.74, 95% CI 1.00, 3.03), CXCL-6 (OR=1.75, 95% CI 1.02, 3.01) and decreased PPT (OR=3.97, 95% CI 2.22, 7.12) were associated with multiple compared to no sites of pain. Biomarker HA (OR=1.57, 95% CI 1.06, 2.32) and decreased PPT (OR=3.53, 95% CI 1.81, 6.88) were associated with multiple compared to a single site of pain.

Conclusion: Biomarkers of inflammation (HA, OPG, IL-6 and CXCL-6), pain (NPY) and PPT may help to understand the etiology of single and multiple pain sites.

Keywords: epidemiology, pain, musculoskeletal, population health

Introduction

Musculoskeletal pain is a highly prevalent condition and considered the largest source of disability worldwide.[1–3] Research has shown individuals with specific sites of musculoskeletal pain are at increased risk of multiple sites of pain or exhibiting higher pain sensitivity, although there remains a lack of understanding on the relationship and development of pain at other sites.[4,5] Increased number of pain sites is associated with worse physical, mental, and emotional functioning, as well as increased economic burden for both patients and health care systems.[2,6–9] Moreover, musculoskeletal pain is complicated by chronic conditions, such as cardiovascular disease (CVD), diabetes (DM), and obesity, though evidence is lacking on how the association occurs, particularly in multiple sites of pain.[10] Other risk factors and conditions for multiple sites of pain include older age, female sex, higher body mass index (BMI), lower physical activity measures, lower education, depression and anxiety.[9,11,12]

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As a growing number of individuals report musculoskeletal pain at more than one site, a better understanding of biologic contributors to pain may be necessary to inform pain management strategies.[13–15] Recently, biochemical markers have been increasingly investigated for their role in chronic pain and heightened pain perception.[16] Inflammatory biomarkers, including interleukin-6 (IL-6), hyaluronan acid (HA), C-C Motif Chemokine Ligand 5 (RANTES), osteoprotegerin (OPG), and C-X-C Motif Chemokine Ligand 6 (CXCL-6), have all been associated with pain intensity and more physical limitations found in chronic pain states.[17–21] Biochemical markers may provide additional information useful for understanding the experience of individual or multiple sites of pain, particularly when adjusting for common chronic comorbidities. There is a lack of research that examines the associations of demographics, individual level characteristics (including common comorbidities like obesity), and inflammatory biomarkers with characteristics of individual site or multiple sites of pain. More research is needed to better understand the associations between individual sites of pain and pain at multiple sites, especially examining these relationships within the community since, historically, the majority of pain-related studies sample primarily from care-seeking populations.

To our knowledge, no other community-based study has examined the relationship between biochemical biomarkers with known associations with inflammation and pain/stress, and multiple sites of pain. As such, the purposes of this study were to: 1) describe the frequency of specific (individual) and multiple pain sites within a community-based cohort study; and 2) determine the association between demographic, individual level characteristics, and biochemical markers with specific as well as multiple sites of pain. We chose inflammatory biomarkers IL-6, HA, RANTES, OPG, and CXCL-6 as they are commonly studied in pain and chronic pain states, including chronic neuropathic pain, low back pain, fibromyalgia and osteoarthritis.[17,18,21,22] We also chose biomarkers NPY and BDNF, which are common biomarkers for pain/stress, secondary to known associations with pain regulation and modulation, especially with the structural degradation associated with low back pain and chronic pain.[22–24] We hypothesize that these certain biochemical biomarkers will be associated with multiple pain sites, regardless of the anatomical location (ie, common factor), and there will be differences among the demographic and individual level characteristics for different individual sites of pain. In addition, we explored factors that were specific to a given anatomical location in order to provide context for the multiple pain site findings and to generate hypotheses for future studies. Although our primary intention was identification of biomarkers associated with pain at single and multiple sites, we also adjusted for other health-related factors, such as medical comorbidities and social determinants of health. This provided an analysis plan to more fully inform our understanding of risk factors associated with pain distribution in a sample of community-based adults.

Methods

The data for this cross-sectional study were collected as part of the Johnston County Osteoarthritis Project (JoCoOA). The JoCoOA project aimed to determine the incidence, prevalence and progression of osteoarthritis among Black and White men and women 45+ years old in a community-based sample ascertained from six townships of Johnston County, North Carolina. Information regarding the sampling strategy and recruitment methods have been described in previous JoCoOA publications.[25] Briefly, JoCoOA participants were selected from the community independently of reports of pain site(s), diagnosis of OA, and biomarker status. Participants were enrolled and data were collected via interviews and clinical examinations during 1991–1997 and 2003–2004; individuals who identified as Black were over-sampled. Approximately every 5 years following initial enrollment, follow-up assessments were performed. Data for the present study were collected from 1697 participants during the second follow-up study visit (2006–2010). All participants in JoCoOA have provided informed consent for participation, and JoCoOA has been continuously approved by the institutional review boards of the University of North Carolina and the Centers for Disease Control and Prevention, which conforms to the Declaration of Helsinki.

Pain Outcomes

Outcomes for these analyses were generated from self-reported pain at multiple locations throughout the body during the clinical examination. Presence of pain at each body location was collected at clinical interview by asking participants to answer "yes" or "no" to the question, "On most days, do you have pain, aching or stiffness in your [neck, upper back/thoracic, low back, shoulder, elbow, wrist, hand, hip, knee, ankle, foot]?" For sites that have bilateral options, such as right and left knee, we counted reports of pain at either or both joints as one site of pain. Sites combined for analysis due to sparse counts of pain included: elbow/wrist/

hand, neck/upper back/thoracic, and ankle/foot. We examined two different outcome categories; individual pain (ie, reporting "yes" in one particular body location) and multiple pain sites (ie, reporting "yes" in more than one body location without regard to a specific body site location). Multiple pain sites were further quantified by summing from 0 to 7. We then categorized this into a single variable of no pain sites (0), single pain site (1) and multiple pain sites (2–7). Other studies have generally categorized multiple sites of pain as 3 or more sites to mirror the definition of chronic widespread pain; however, in this analysis we used more than one site of pain as the criterion for having multiple sites of pain to reflect increasing number of pain sites without regard for anatomic relationship.[26,27] Including individuals without pain at one or more sites allows for a comparison that is not often an option, as most studies include only care-seeking cohorts.

Exposures

Demographic and Individual Level Characteristics

Demographic information including age, gender (male/female), race (Black/White) and educational status (<high school, high school, >high school) were collected by self-report. Age, gender, race, and educational status are traditional demographic features included in studies examining pain; these variables have shown associations with multiple sites of pain in previous research.[7,9,11,12]

Comorbidities

Body mass index (BMI in kg/m^2) was calculated at the time of clinical examination using height without shoes and weight as measured on a balance beam scale. Those with a BMI $\geq$ 30.0 kg/m^2 were categorized as obese. The covariate DM was self-reported by asking participants a "yes" or "no" question if they had ever been diagnosed with "diabetes or high blood sugar." CVD included self-reports of heart attack, angina or angina pectoris, congestive heart failure, other heart problems, cerebrovascular accident, and peripheral vascular disease. Diabetes, obesity, and CVD were included in the analysis due to the known associations with musculoskeletal pain, particularly increased number of pain sites.[10,28–30]

Serum Biochemical Biomarkers

Details regarding collection of biospecimens, as well as the participants with biochemical samples have been described elsewhere.[31] All samples were collected after completion of morning activity at a time (>1 hour after arising) when these serum markers have attained equilibrium. The biomarkers associated with inflammation selected for this study included interleukin-6 (IL-6), hyaluronan (HA), C-C Motif Chemokine Ligand 5 (RANTES), osteoprotegerin (OPG), and C-X-C Motif Chemokine Ligand 6 (CXCL-6). Serum biomarkers were measured in singlicate as our prior duplicate pilot work has demonstrated excellent reliability and validity (coefficient of variation (CV) below 15% representing good reliability).[22] A human control serum sample was quantified in duplicate on all plates to determine intra- (within) and inter- (between) assay coefficients of variation. Supplementary Table 1 includes additional information pertaining to the biomarkers including: biomarker with details of the manufacturer, dilution, means and distributions, intra-and inter-assay coefficients of variation. All samples had concentrations in the detectable range for all biomarkers.

Pressure Pain Threshold

Pressure pain threshold (PPT) is a well-validated measure for pain sensitivity.[32] PPT was conducted using a standard mechanical pressure-based dolorimeter (measured in kilograms of pressure) at bilateral upper trapezius sites. All PPT clinical measurements were performed by a single research assistant utilizing an a-priori measurement protocol that has shown good reliability.[32] The protocol started with a "practice trial" to show the device to the participant. Following the practice trial, pressure was applied to the upper trapezius at a rate of 1 kg per second until the patient reported pain, or until a maximum pressure of 4 kg was achieved without a report of pain (reported as ">4.0 kg"). Trials were repeated until two consecutive readings were within 0.4 kg, with a maximum of four trials per side. This procedure was performed first on the left trapezius, then on the right for a total of two measurements that were averaged into a single PPT score.[33] This protocol results in a dichotomous measure of PPT with a score of < 4.0 kg indicating a higher pain sensitivity.

Statistical Analysis

Means with standard deviations, medians and ranges were used to describe the distributions of biomarkers. Intra- and inter-assay CVs were used to describe the reliability of each assay; CVs less than 15% represented assays with good reliability. The lower limit of detection for each biomarker, quantification range, and the dilution used for each assay are also reported. Differences with respect to the absence and presence of each outcome were conducted using *t*-tests and chi-square tests as appropriate. Due to skewness, each biomarker was natural log-transformed prior to conducting regression analysis.

Descriptive statistics were used to summarize study subjects and all relevant variables. Counts and percentages were produced for categorical variables, while mean and standard deviation were computed for continuous variables. To assess associations of covariates with presence of pain by site, multivariable logistic regression was used to separately model the log odds of presence of pain at each of seven sites: 1) Neck/Upper Back/Thoracic; 2) Lower Back; 3) Hip(s); 4) Knee(s); 5) Foot/Ankle(s); 6) Shoulder(s); and 7) Elbow/Wrist/Hand(s). To assess associations of covariables with presence of pain at no sites, a single site, or multiple sites, two models were used: 1) Treating the three-level outcome of pain at no sites, a single site, or multiple sites as nominal, for which a multivariable generalized logits regression model was used; 2) Treating the three-level outcome as ordinal, for which a multivariable proportional odds model using cumulative logits was used.

Models included all previously defined demographic, comorbidity, and biomarker variables to produce adjusted odds ratios (aOR) and corresponding 95% confidence intervals (CIs). Complete cases were included for multivariable analyses, excluding subjects with missing covariates given that missing data (7.5%) was well below <10% (Figure 1) among participants with available serum data.

All analyses were performed with SAS version 9.4 (SAS institute Inc., Cary, NC). The significance level for these analyses was determined at 0.05.

Results

An illustration of the selection of the sample used for these analyses can be found in Figure 1. Of the 1697 participants that entered the second follow-up time point, 1686 (99.4%) had complete pain data. Based on the availability and volume of serum biospecimens remaining, biochemical analyses were conducted on a subsample of 689 (40.6%) individuals.

The demographics of the sample and outcome characteristics are presented in Table 1. The average age was 66.0. The majority of participants were women (69.5%) and 33.4% of participants identified as Black. The average BMI was 31.5 ± 6.1 kg/

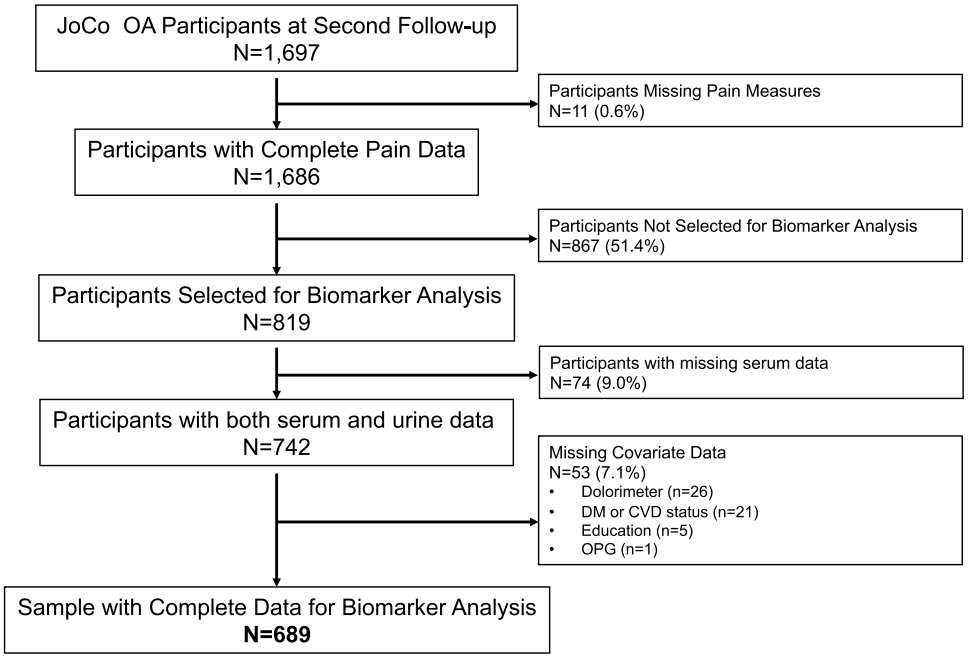

Figure 1 Flow Diagram of Study Participants.

Table 1 Demographics and Clinical Characteristics of Biomarker Subsample with Complete Data, N=689

	Overall (N=689)
Covariables	
Age (range=50–88), years (mean ± SD)	66.0 ± 7.4
Gender, n (%)	
Male	210 (30.5%)
Female	479 (69.5%)
Race, n (%)	
Black	230 (33.4%)
White	459 (66.6%)
Education, n (%)	
Less than High School	91 (13.2%)
High School	341 (49.5%)
More than High school	257 (37.3%)
Comorbidities	
BMI (range=18–55), kg/m^2 (mean ± SD)	31.5 ± 6.1
Obese (≥ 30 kg/m^2, n (%))	373 (54.1%)
CVD, n (%)	158 (22.9%)
DM, n (%)	135 (19.6%)
Biomarkers (log transformed)	
OPG (range=2.2–5.7), ln(pg/mL) (mean ± SD)	4.5 ± 0.4
CXCL-6 (range=4.2–9.8), ln(pg/mL) (mean ± SD)	5.4 ± 0.5
RANTES (range=10–13), ln(pg/mL) (mean ± SD)	12.0 ± 0.5
IL-6 (range=−1.3–4.4), ln(pg/mL) (mean ± SD)	0.3 ± 0.7
HA (range-2.1–7.5), ln(ng/mL) (mean ± SD)	4.0 ± 0.7
BDNF (range=9–11), ln(pg/mL) (mean ± SD)	10.3 ± 0.3
NPY (range=1.1–4.5), ln(pg/mL) (mean ± SD)	3.1 ± 0.4
Dolorimeter (range=0.8–4.0), kg mean ± SD)	3.7 ± 0.7
Pressure pain sensitivity (<4.0 kg/s, n (%))	193 (28.0%)
Outcomes	
Anatomic Pain Site, n (%)	
Neck/Upper Back/Thoracic	231 (33.6%)
Lower Back	316 (45.9%)
Hip(s)	259 (37.6%)
Knee (s)	291 (42.3%)
Foot/Ankle (s)	230 (33.4%)
Shoulder (s)	214 (31.1%)
Elbow/Wrist/Hand (s)	326 (47.4%)
Sum of Pain Sites, n (%)	
0	158 (23.0%)
1	95 (13.8%)
2	92 (13.4%)
3	98 (14.2%)
4	79 (11.5%)
5	63 (9.2%)
6	58 (8.4%)
7	45 (6.5%)
2+ Pain Sites	435 (63.2%)

Abbreviations: BMI, Body Mass Index; DM, diabetes mellitus; CVD, cardiovascular disease; OPG, osteoprotegerin, CXCL6, C-X-C Motif Chemokine Ligand 6; RANTES, C-C Motif Chemokine 5; IL-6, interleukin-6; HA, hyaluronan; BDNF, brain derived neurotrophic factor; NPY, neuropeptide-Y.

m^2, with over half (54.1%) of participants categorized as obese. Twenty-three percent of participants reported having no pain at any of the body sites, approximately 14% had pain at one body site, and 63% had pain at two or more body sites. The most common site of pain was the elbow/wrist/hand (47.4%), closely followed by the lower back (45.9%).

Results for the different demographic and individual level characteristics, and biomarker data at each individual pain site are presented in Table 2, adjusted for age, gender, race, education, the comorbidities of CVD, DM, and obesity. Generally, Black individuals reported less sites of pain, while women and those with lower education reported more pain in the included sites. Obesity and CVD were both associated with higher odds of pain at the low back, hip(s), knee(s), foot/ankle(s) compared to individuals without obesity or CVD. There were higher odds of reporting shoulder pain (OR=1.77, 95% CI 1.21 to 2.59) for obese individuals compared to non-obese individuals. Odds of elbow/wrist/hand pain (OR=1.77, 95% CI 1.15 to 2.74) were higher in those reporting DM compared to those not reporting DM. Increased serum concentration of IL-6 and HA were associated with higher odds of knee pain (OR=1.30, 95% CI 1.03 to 1.64 and OR=1.32, 95% CI 1.01 to 1.73, respectively). Increased serum concentration of HA was also associated with higher odds of elbow/wrist/hand pain (OR=1.60, 95% CI 1.22 to 2.09), while increased serum concentration of NPY was associated with lower odds of shoulder pain (OR=0.56, 95% CI 0.33, 0.93). Those with a PPT <4.0 kg indicating higher pain sensitivity, had higher odds of reporting pain for every site included in this study.

Table 3 describes the relationships between predictors and no, single, and/or multiple sites of pain. Generally, participants who identified as Black had lower odds of reporting pain compared to those who identified as White; while individuals with obesity had higher odds of reporting pain compared to non-obese individuals. Participants with increased serum levels of OPG (OR=1.74, 95% CI 1.00 to 3.03), CXCL-6 (OR=1.75, 95% CI 1.02 to 3.01), and HA (OR=1.50, 95% CI 1.07 to 2.10) had higher odds of reporting multiple sites of pain compared to no pain sites. Participants with increased serum levels of HA also had higher odds (OR=1.57, 95% CI 1.06 to 3.32) of having multiple sites of pain compared to a single site of pain. Participants with higher pain sensitivity on the PPT (<4 kg) had 3 to 4 times higher odds of reporting multiple sites of pain compared to no or single sites of pain. Table 3 also describes the proportional odds cumulated over no, individual, and multiple sites of pain. Largely, participants who identified as Black had lower odds of greater number of pain sites versus no sites of pain, and obese participants had higher odds of greater number of pain sites versus no sites of pain. Participants with increased serum levels of OPG (OR=1.58, 95% CI 1.01 to 2.47), CXCL-6 (OR=1.64, 95% CI 1.06 to 2.55), HA (OR=1.45, 95% CI 1.10 to 1.90), and lower PPT (higher pain sensitivity, OR=3.78, 95% CI 2.39 to 5.99) had higher odds of reporting pain as the number of pain sites increased.

Discussion

In this analysis, we investigated associations between demographics, individual level characteristics, and biochemical biomarkers for specific and multiple pain sites. To the best of our knowledge, this is one of the first community-based studies within the United States to examine these relationships. This unique, community-based sample was diverse and offered a variety of plausible biological markers for analysis of biomarker associations with pain sites. These findings offer valuable information regarding pain distributions to complement information provided by cohorts of patients seeking care for musculoskeletal pain conditions. In this community-based cohort, the majority of participants reported multiple sites of pain, while only a quarter reported no pain, and even fewer reported a single site of pain.

One objective during this analysis was to evaluate the frequency of specific and multiple pain sites within a community-based cohort. Our results related to the sum of pain sites are consistent with other studies that found a majority of people reported multiple sites of pain. Roughly two-thirds of the sample reported 2 or more sites of pain, which is slightly higher than the prevalence of multi-site pain in the general public.[15] This increased prevalence may have been influenced by the older average age of our population (66 years old) compared to the general population. Consistent with our results, a study examining adults over the age of 65, observed that approximately 75% of adults reported multiple sites of pain.[34] These findings indicate increased musculoskeletal pain seen in aging population, which could contribute to functional limitations or disability.[35,36] Participants who identified as Black demonstrated reduced odds of pain at most of the individual sites, as well as reduced odds of reporting both single and multiple sites of pain compared to White participants. Literature suggests that pain is underreported in individuals who identify as Black although pain severity tends to be higher in this population.[37] Due to the nature of the data collection, we are unable to

Table 2 Cross-Sectional Associations* Between Presence of Individual Pain Sites with Demographics, Comorbidities, and Biomarkers, N=689

Covariables	**Outcomes**						
	Neck/Upper Back/Thoracic	**Lower Back**	**Hip(s)**	**Knee(s)**	**Foot/ Ankle (s)**	**Shoulder(s)**	**Elbow/Wrist/ Hand (s)**
	aOR (95% CI)	***aOR (95% CI)***	***aOR (95% CI)***	***aOR (95% CI)***	***aOR (95% CI)***	***aOR (95% CI)***	***aOR (95% CI)***
DEMOGRAPHICS							
Age (1 year increase)	0.99 (0.96, 1.01)	0.98 (0.96, 1.01)	**0.97 (0.95, 0.99)**	0.98 (0.96, 1.01)	0.98 (0.95, 1.01)	0.97 (0.94, 1.00)	**0.97 (0.94, 0.99)**
Gender (F vs M)	**1.58 (1.04, 2.40)**	1.16 (0.79, 1.69)	1.21 (0.82, 1.80)	1.35 (0.91, 2.00)	**2.06 (1.34, 3.18)**	1.17 (0.76, 1.79)	**1.71 (1.16, 2.51)**
Race (B vs W)	**0.45 (0.27, 0.76)**	**0.43 (0.26, 0.70)**	**0.52 (0.31, 0.85)**	0.76 (0.47, 1.24)	**0.42 (0.25, 0.72)**	0.66 (0.39, 1.12)	**0.48 (0.29, 0.78)**
Education: HS vs >HS	1.37 (0.93, 2.01)	1.19 (0.83, 1.71)	1.10 (0.76, 1.59)	1.08 (0.75, 1.57)	1.00 (0.68, 1.48)	1.15 (0.77, 1.71)	0.99 (0.69, 1.42)
Education: <HS vs >HS	**2.28 (1.32, 3.93)**	**2.20 (1.28, 3.77)**	**1.83 (1.08, 3.12)**	1.31 (0.76, 2.24)	**1.95 (1.13, 3.36)**	**1.78 (1.02, 3.11)**	1.57 (0.91, 2.70)
COMORBIDITIES							
Obesity	1.09 (0.75, 1.57)	**1.67 (1.18, 2.35)**	**1.44 (1.01, 2.05)**	**2.11 (1.48, 2.99)**	**1.51 (1.04, 2.19)**	**1.77 (1.21, 2.59)**	1.20 (0.85, 1.71)
CVD	1.23 (0.83, 1.84)	**1.67 (1.13, 2.46)**	**1.48 (1.01, 2.18)**	**1.51 (1.02, 2.23)**	**1.53 (1.03, 2.28)**	1.16 (0.77, 1.74)	1.23 (0.84, 1.82)
DM	1.23 (0.79, 1.91)	1.15 (0.75, 1.76)	1.26 (0.82, 1.93)	1.18 (0.77, 1.81)	1.30 (0.84, 2.03)	1.19 (0.76, 1.85)	**1.77 (1.15, 2.74)**
BIOMARKERS (for a 1 unit increase in ln(biomarker))							
OPG, ln(pg/mL)	1.20 (0.75, 1.90)	1.29 (0.84, 2.00)	1.53 (0.98, 2.41)	1.10 (0.70, 1.71)	0.69 (0.43, 1.09)	1.07 (0.67, 1.71)	1.35 (0.87, 2.10)
CXCL6, ln(pg/mL)	1.18 (0.76, 1.82)	1.30 (0.86, 1.97)	1.03 (0.67, 1.57)	1.16 (0.76, 1.77)	1.34 (0.86, 2.07)	1.14 (0.73, 1.80)	1.28 (0.84, 1.96)
RANTES, ln(pg/mL)	0.98 (0.64, 1.50)	1.01 (0.68, 1.51)	1.35 (0.89, 2.05)	1.22 (0.81, 1.84)	1.45 (0.93, 2.25)	1.24 (0.80, 1.93)	0.92 (0.61, 1.38)
IL-6, ln(pg/mL)	1.13 (0.89, 1.44)	1.08 (0.86, 1.36)	0.97 (0.76, 1.22)	**1.30 (1.03, 1.64)**	1.14 (0.89, 1.46)	1.15 (0.90, 1.48)	1.04 (0.82, 1.31)
HA, ln(ng/mL)	1.02 (0.78, 1.34)	1.10 (0.85, 1.43)	1.10 (0.85, 1.43)	**1.32 (1.01, 1.73)**	1.14 (0.87, 1.51)	1.14 (0.86, 1.51)	**1.60 (1.22, 2.09)**
BDNF, ln(pg/mL)	0.95 (0.50, 1.82)	0.71 (0.39, 1.31)	0.70 (0.37, 1.31)	0.60 (0.32, 1.13)	0.77 (0.40, 1.48)	0.72 (0.37, 1.39)	1.02 (0.55, 1.89)
NPY, ln(pg/mL)	0.78 (0.47, 1.28)	0.80 (0.50, 1.28)	0.81 (0.50, 1.32)	0.66 (0.41, 1.08)	0.80 (0.49, 1.32)	**0.56 (0.33, 0.93)**	0.66 (0.41, 1.07)
Pressure pain sensitivity: Dolorimeter <4 vs 4 kg/s	**3.10 (2.12, 4.54)**	**2.35 (1.61, 3.43)**	**2.44 (1.68, 3.56)**	**2.46 (1.68, 3.61)**	**2.53 (1.72, 3.70)**	**3.13 (2.12, 4.62)**	**2.76 (1.87, 4.06)**

Notes: *Results from seven, separate by site, multivariable logistic regression models for dichotomous presence of pain; aOR=Odds Ratio, represents the association between each covariate and the outcome while simultaneously adjusted for all other explanatory variables in the table; 95% CI= 95% Confidence Interval; **Bold** values indicate a 95% CI where the null effect of OR=1 is not included, at a 0.05 level of significance.

Abbreviations: F, Female; M, Male; B, Black; W, White; HS, High School Education; >HS, more than high school education; <HS, less than high school education; Obesity, Body Mass Index≥30 kg/m^2; DM, diabetes mellitus; CVD, cardiovascular disease; OPG, osteoprotegerin; CXCL6, C-X-C Motif Chemokine Ligand 6; RANTES, C-C Motif Chemokine 5; IL-6, interleukin-6; HA, hyaluronan; BDNF, brain derived neurotrophic factor; NPY, neuropeptide-Y.

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Table 3 Cross-Sectional Associations* Between Multiple, Single and No Pain Sites with Demographics, Comorbidities, and Biomarkers, N=689

Covariables	**Generalized Logits Model Treating Three-Level Outcome as Nominal**			**Proportional Odds Model Using Cumulative Logits Treating Three-Level Outcome as Ordinal**
	Single Site of Pain vs No Pain	**Multiple Sites of Pain vs No Pain**	**Multiple Sites of Pain vs Single Site of Pain**	**Cumulated Over Multiple to No Sites of Pain**
	aOR (95% CI)	***aOR (95% CI)***	***aOR (95% CI)***	***aOR (95% CI)***
DEMOGRAPHICS				
Age (1 year increase)	0.97 (0.93, 1.01)	0.97 (0.94, 1.00)	1.00 (0.96, 1.03)	0.98 (0.95, 1.00)
Gender (F vs M)	1.17 (0.66, 2.06)	1.51 (0.97, 2.36)	1.30 (0.77, 2.17)	1.40 (0.97, 2.01)
Race (B vs W)	**0.33 (0.15, 0.73)**	**0.25 (0.13, 0.45)**	0.76 (0.37, 1.57)	**0.33 (0.20, 0.54)**
Education: HS vs >HS	0.89 (0.50, 1.57)	1.11 (0.71, 1.72)	1.25 (0.75, 2.08)	1.11 (0.78, 1.59)
Education: <HS vs >HS	1.39 (0.53, 3.68)	1.86 (0.88, 3.96)	1.34 (0.60, 2.99)	1.67 (0.93, 3.02)
COMORBIDITIES				
Obesity	**1.77 (1.00, 3.12)**	**2.34 (1.52, 3.62)**	1.32 (0.81, 2.17)	**2.02 (1.43, 2.87)**
CVD	1.39 (0.69, 2.77)	1.58 (0.93, 2.71)	1.14 (0.64, 2.03)	1.40 (0.92, 2.13)
DM	1.03 (0.45, 2.31)	1.60 (0.88, 2.91)	1.56 (0.79, 3.06)	1.51 (0.94, 2.43)
BIOMARKERS (for a 1 unit increase in ln(biomarker))				
OPG, ln(pg/mL)	1.36 (0.67, 2.73)	**1.74 (1.00, 3.03)**	1.28 (0.69, 2.38)	**1.58 (1.01, 2.47)**
CXCL6, ln(pg/mL)	1.22 (0.74, 2.00)	**1.75 (1.02, 3.01)**	1.21 (0.65, 2.25)	**1.64 (1.06, 2.55)**
RANTES, ln(pg/mL)	1.26 (0.66, 2.39)	1.22 (0.74, 2.00)	0.97 (0.54, 1.72)	1.18 (0.79, 1.76)
IL-6, ln(pg/mL)	1.10 (0.75, 1.60)	1.08 (0.81, 1.44)	0.99 (0.71, 1.38)	1.02 (0.81, 1.29)
HA, ln(ng/mL)	0.95 (0.61, 1.48)	**1.50 (1.07, 2.10)**	**1.57 (1.06, 2.32)**	**1.45 (1.10, 1.90)**
BDNF, ln(pg/mL)	0.61 (0.23, 1.63)	0.64 (0.30, 1.37)	1.06 (0.44, 2.55)	0.69 (0.37, 1.28)
NPY, ln(pg/mL)	0.89 (0.42, 1.88)	0.63 (0.35, 1.15)	0.72 (0.36, 1.41)	0.69 (0.43, 1.11)
Pressure pain sensitivity: Dolorimeter <4 vs 4 kg/s	1.13 (0.49, 2.57)	**3.97 (2.22, 7.12)**	**3.53 (1.81, 6.88)**	**3.78 (2.39, 5.99)**

Notes: *Results from a multivariable generalized logits model and a multivariable cumulative logits model for a three-level, polytomous outcome of no pain, single site pain, and multiple site pain; aOR=Odds Ratio, represents the association between each covariate and the outcome while simultaneously adjusted for all other explanatory variables in the table; 95% CI= 95% Confidence Interval; Bold values indicate a 95% CI where the null effect of OR=1 is not included, at a 0.05 level of significance.
Abbreviations: F, Female; M, Male; B, Black; W, White; HS, High School Education, >HS, more than high school education; <HS, less than high school education; Obesity, Body Mass Index>30 kg/m^2; DM, diabetes mellitus; CVD, cardiovascular disease; OPG, osteoprotegerin; CXCL6, C-X-C Motif Chemokine Ligand 6; RANTES, C-C Motif Chemokine 5; IL-6, interleukin-6; HA, hyaluronan; BDNF, brain derived neurotrophic factor; NPY, neuropeptide-Y.

determine if participants who identify as Black in the JoCoOA sample underreport their pain or truly have less sites of pain. Female gender and lower educational status were also significantly associated with individual sites of pain, but these demographic features were not determined to be statistically significant for single and multiple site pain analyses.

Overwhelmingly, the most consistent factor in individual and multiple pain sites was the PPT, as it was statistically significant for every individual site analysis. Furthermore, those with multiple pain sites had significantly lower PPT thresholds (higher pain sensitivity) than those without pain or only a single site pain; however, there was no significant difference in PPT for single site compared to no pain. This finding indicates that those experiencing multiple pain sites may have increased pain sensitivity and altered pain processing.[38] Increased pain sensitivity often leads to worse outcomes and additional healthcare needs, so it is vital to identify objective measures to facilitate identification of individuals experiencing altered pain processing early in order to target appropriate treatment interventions.[39] This nonclinical, population-based sample combined with PPT as a form of quantitative sensory testing allows for improved understanding of musculoskeletal pain and pain sensitivity.[40] Use of PPT in the clinical setting is feasible as it is a low-cost application with high user and patient acceptability.[32,41,42] Thus, use in the clinical setting with further development and understanding of PPT thresholds may help to identify individuals at risk for multiple sites of musculoskeletal pain.[42] A deeper understanding of how pain sensitivity

contributes to perceived functional limitations will also improve clinical management and outcomes of patients with multiple sites of pain. Given the unique design of examining association with presence of pain and quantitative sensory testing in the form of PPT, we are contributing to an understanding of identification of potential risk factors for pain in a population-based cohort. Our results corroborate that lower PPT thresholds may identify patients experiencing altered pain processing to develop means of addressing their altered pain perceptions early, and even in community-based samples this higher pain sensitivity may be linked with having more sites of pain.

Even after accounting for PPT, we identified two biochemical biomarkers that were associated with multiple pain sites (OPG and CXCL-6), indicating potential roles of these biomarkers in more complex, multiple sites of pain. Higher levels of CXCL-6 have been associated with chronic neuropathic pain, and moderate to severe low back pain, implicating this biochemical marker in possible pain pathways.[21,43] The biomarker OPG is a neuropeptide that has been shown to be related to neurogenic inflammation, especially in conditions such as complex regional pain syndrome, which may contribute to mechanisms for multiple sites of pain.[44] Our findings showed elevated HA levels were seen in both multiple sites of pain and individual pain sites. Increased levels of biomarker HA have been seen in patients with OA, particularly in the spine, hip, and knee joints; previous studies indicate that elevated levels of HA increase with higher levels of OA burden due to the role of HA in inflammatory pathways associated with symptoms of increased stiffness and swelling.[45,46] Additionally, our results demonstrated an association of HA with elbow/wrist/hand pain which is consistent with previous research demonstrating elevated HA levels are associated with increased number of arthritic joints, especially in the hand, which can contribute to increased pain and stiffness.[47–49] We found IL-6 and NPY to be associated with specific sites of pain, knee and shoulder pain respectively. Biomarker IL-6 is a cytokine, and has been found to play a role as an inflammatory mediator in several pain pathways which can exacerbate pain.[50] NPY has also been shown to play a role in pain perception, specifically in low back pain and chronic pain, but there is little research on the role of NPY, shoulder pain and OA symptoms.[22,51] This cross-sectional analysis corroborates the hypothesis that biomarkers may play a role in both individual and multiple sites of pain. As such, our analyses support the further examination of the utility of biochemical biomarkers as objective indicators of individual pain sites or multiple sites of pain.

Several of the biomarkers associated with pain are also known to be associated with other comorbid, chronic conditions. Although we adjusted for the common comorbidities of DM, CVD, and obesity, since this was a cross-sectional analysis, we cannot determine a temporal or causal component of these conditions on the development of pain at individual or multiple sites. These conditions are hypothesized to be associated with multiple sites of pain due compounding issues which contribute to comorbidities and pain such as poor diet, systemic inflammation, and altered ability to participate in physical activity.[28–30]Our results show those with obesity have almost double the odds of having multiple sites pain compared to those with single site and no pain. Obesity and multiple sites of pain have been shown to have a strong cross-sectional association, particularly in OA-related pain due to increased mechanical loads on joints secondary to increased body mass.[6,7,12,52] Altered biomarker levels, including IL-6, can signify dysregulation of inflammatory systems, which has been shown to be associated with increased pain and obesity in certain populations.[53,54] Elevated BMI is associated with chronic inflammation and pain, which could be explained by biomarkers included in this study.[55] Again, our data are cross-sectional so we cannot determine a temporal relationship, though our results indicate further analysis of biomarkers in contribution to systemic inflammation which may exacerbate conditions of pain and/or obesity. Evidence also supports that musculoskeletal pain, especially OA pain, is associated with CVD, and individuals with increased number of pain sites are known to have an increased risk of cardiovascular death.[28,30] Finally, a longitudinal study showed that patients with diabetes have a higher cumulative-incidence of musculoskeletal pain compared to non-diabetic groups over a 10-year period.[29] These conditions, especially obesity, are hypothesized to be linked with musculoskeletal pain through inflammatory pathways (ie, metabolic syndrome) which can also exacerbate conditions such as DM and CVD.[54,56]

Our study has several strengths including the fact that it is a community-based study, examining the associations among demographic, individual level characteristics, and biochemical markers, and individual as well as multiple sites of pain. Our large sample size of participants of Black or White race of both genders allowed for several comparisons within the analyses. As such, these analyses may be more generalizable to the population outside of the more commonly studied clinically based cohorts by providing information from a community-based sample.[58] This furthers our understanding of pain sensitivity and pain patterns in a larger population which has been identified as a critical gap in the pain research literature.[40,57] However, this study does have several limitations. Despite the fact that our biomarker findings were consistent with other studies that found

correlations with these biomarkers and musculoskeletal pain, several biomarkers included in this study did not demonstrate an association with individual pain sites or multiple sites of pain. The lack of significant findings for several biomarkers could be attributed to the fact that pain was defined as dichotomous (yes/no), whereas other biomarker studies use a continuum of pain intensity ratings, duration, or other measures as the definition of pain or pain interference.[17,18,24,47] Another limitation relates to the cross-sectional design of this study resulting in our only being able to study the association between biomarkers and individual sites of pain or multiple sites of pain, not the causal relationships between these biomarkers and the development of multiple sites of pain. Additionally, biochemical markers were only measured on a subset of the population, and therefore may not be representative of the entire cohort. Our measurement of PPT was dichotomized per the protocol of this epidemiologic study differs from PPT values which can also be treated as continuous. Defining a cutoff of PPT for pain sensitivity may be more pragmatic and useful to identify patients at risk for increased pain sensitivity. Finally, we collected comorbidities from participant self-report on DM and CVD which may be prone to measurement error. In addition, we did not have information on participants' history of trauma, surgery, or other conditions which might contribute to pain. Future studies are warranted to examine whether these biochemical biomarkers may predict the progression from single to multiple sites of body pain.

In summary, community members having multiple pain sites are more likely to have higher pain sensitivity and have associations with certain biochemical markers, especially OPG, CXCL-6, and HA. In contrast, there were two biochemical markers (IL-6 and NPY), that were only associated with specific pain sites. These findings support our goal of identifying biochemical markers, particularly those related to inflammation, that may aid in objectively distinguishing risk factors for pain that progresses from one to multiple sites. Combining these findings with other clinical outcomes, and potentially diagnostic imaging, may provide further insight into the development of chronic, multi-site pain, and associated disabilities.

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Disclosure

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