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Associations between the chemokine biomarker CCL2 and knee osteoarthritis outcomes: The Johnston County Osteoarthritis Project

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Abstract

Objective—Our study analyzes the association between chemokine-ligand-2 (CCL2) serum concentrations at baseline and knee radiographic OA (knee-rOA), knee-rOA progression, individual radiographic features and knee symptomatic OA at 5-year follow-up.

Design—OA outcomes were analyzed in a community-based cohort including a baseline enrollment and a 5-year follow-up. Baseline CCL2 serum concentrations were assessed by

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Author Contribution

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multiplex assay and associated with presence or progression of individual radiographic features at 5-year follow-up. Separate multiple logistic regression models were used to examine adjusted associations between baseline CCL2 and each of the knee OA variables at follow-up. CCL2 at baseline was modeled as an explanatory variable, whereas each of the knee OA variables at follow-up served as the response variables. Models were adjusted for age, BMI, race, and sex. Trend tests were conducted to assess any linear effect on outcomes across CCL2 tertiles.

Results—Participants (n=168) had a median age of 57-years and median BMI of 29 kg/m^2. About 63% of all participants were women, and 58% Caucasian (42% African American). In adjusted logistic models, continuous log-CCL2 was significantly associated with knee-rOA. For each unit increase in log CCL2, the odds of having knee-rOA at follow-up was increased by 72%. CCL2 tertiles showed significant linear associations with presence and progression of knee-rOA and medial joint space narrowing, but not with presence or progression of osteophytes, bone sclerosis, knee symptoms, or symptomatic knee-rOA.

Conclusions—Serum CCL2 may help to elucidate some mechanisms of joint destruction and identify individuals with higher odds of structural knee changes.

Keywords

Chemokines; Osteoarthritis; Biomarkers

INTRODUCTION

Chemokines are mediators of the disrupted joint tissue metabolism in osteoarthritis (OA) [1]. The chemokine-ligand-2 (CCL2), ligand of C-C-chemokines-receptor-2 (CCR2), is expressed in chondrocytes, osteoblasts and synovial cells[1] and has a role in bone metabolism and OA[2] [3]. CCR2+ cells are abundant in human OA synovium and CCR2+ macrophages are associated with OA cartilage damage[2]. Our previous murine studies demonstrated that CCR2 mediates OA cartilage and bone damage, independently from macrophage synovial infiltration[3]. In addition, a study on human chondrocytes demonstrated that CCL2 treatment led to chondrocyte degradation[4]. Rodent studies have shown that CCL2/CCR2 axis mediates OA pain[2, 3], suggesting that increased circulating CCL2 levels during OA might reflect altered bone/cartilage metabolism leading to joint destruction and pain.

Recently, there has been an effort to find biochemical markers that could aid in OA diagnosis, predict progression, and identify individuals who are at higher OA risk, allowing preventive measures to delay the disease. Serum measures represent a minimally-invasive method to measure biomarkers.

Our study uses data from the Johnston County Osteoarthritis Project (JoCoOA), a community-based study among African Americans (AAs) and Caucasians[5]. Specifically, we analyzed associations between CCL2 serum concentrations at baseline and knee-rOA, medial joint space narrowing (JSN), osteophytes, bone sclerosis and symptomatic knee OA, at 5-year follow-up. These data provide insights for CCL2 as potential serum biomarker to evaluate OA risk.

PARTICIPANTS AND METHODS

Sampling/recruitment methods have been previously described, and included a baseline enrollment (1991–98) and a 5-year follow-up[5, 6]. The JoCoOA study has been approved by the Institutional Review Boards of the University of North Carolina and Centers for Disease Control and Prevention.

Participants represent a range of ages, equal proportions of AA and Caucasian women and men, with or without knee-rOA, with available bilateral anterior-posterior radiographic knee data and available non-fasting serum samples at baseline. Radiographs were read without knowledge of participant clinical status by a single musculoskeletal radiologist (JBR) as previously reported[7] with high intra-rater and inter-rater reliability (weighted kappa 0.9) [5]. Radiographic changes were measured using the Kellgren-Lawrence (K/L) grading system[8]. Participants with evidence of Rheumatoid Arthritis were excluded from the study, as well as smokers and subjects who were taking estrogens, due to the impact of cigarette smoking and estrogens on cytokines/chemokines[9, 10]. No participants subjected to knee arthroplasty are included since none fulfilled our inclusion criteria.

Demographic and clinical variables

Race (AAs/Caucasian), sex, age, and baseline body mass index (BMI) were collected as participant characteristics.

Knee Symptoms and Symptomatic OA

Participants answered the following question: "*On most days, do you have pain, aching or stiffness in your [left/right] knee?*". An affirmative answer was considered a positive knee-symptom (Sx). In addition to our binary assessment of knee-Sx, we also examined knee-Sx severity, defined as none, mild, moderate/severe. We also examined the WOMAC Pain Sub-scale, defined continuously and as >= 3 vs 3 or lower[11]. Since this scale is person-based, and not joint-based, we could not use this for defining Symptomatic OA (SxOA), which required evidence of knee-rOA and knee-Sx in the same knee.

Knee Radiographic Assessment

Participants completed bilateral anterior-posterior radiography of both knees with weight-bearing and foot mat positioning at baseline and follow-up. Knee-rOA was defined as having a K/L grade≥2; knee-rOA progression was defined as having at least one unit increase in K/L grade in the same knee from baseline to follow-up, regardless of the K/L grade at baseline (no bilateral K/L=4 were present at baseline).

Other Knee Radiographic Features

We also examined osteophytes and minimal joint space narrowing (JSN). Because JSN is more prevalent in the medial compartment than in the lateral, and semi-quantitative scoring is more accurate in the medial compartment, we restricted our analysis to the medial compartment. This was borne out in our data, with only 14 of 168 showing any JSN in the lateral compartment. Medial osteophytes (tibial and femoral) and JSN were scored semi-quantitatively (0–3) using the Burnett atlas[12]. For osteophytes, the levels were: 0=absent,

1=mild, 2=moderate, 3=severe; the higher score of tibial and femoral sides was used as the overall osteophyte score[7]. For JSN, the levels are 0=normal, 1=up to 33% abnormal, 2=34–66% abnormal; 3=67–100% abnormal[7]. Progression of knee osteophytes or JSN was defined as having at least one unit increase from baseline to follow-up in the overall osteophytes or JSN scores, respectively.

Chemokine Biomarker Assays

Baseline serum CCL2 concentrations were measured using the BioPlex 200 (Bio-Rad) utilizing Fluorokine Multianalyte Profiling (FMAP) Kits (R&D Systems) employing a fluorescently labeled bead-based multiplex assay based on specialized flow cytometry. FMAP Kits are highly efficient reagents, validated for the quantification of factors in complex matrices, and one replicate was used. Monoclonal antibodies are covalently coupled to specific fluorescent beads. Fifteen microliters of each sample were used. Fluorescence intensity (FI) from the immunoassay was analyzed using the Bio-Plex 5.0 software (Bio-Rad, Hercules, CA). Each assay was performed according to reagent manufacturer's protocol. All the FI values fell within the range of the five central standards (S2–S6) and almost all fell between S3 and S5. In all the standard curves (one for each plate) the maximum coefficient of variation (CV) observed for the duplicate standards was 8.5%.

Statistical Analysis

Descriptive statistics were calculated for the sample. We computed the median and range for continuous variables and frequencies and percentages for categorical variables. To assess the impact of missing values, these variables were compared between participants with and without missing values via Fisher's exact and t-tests, as appropriate.

Due to the skewed distribution, a base "e" logarithmic transformation was applied to CCL2 to more closely approximate a normal distribution. We additionally examined CCL2 categorized into tertiles for ease of interpretation.

Separate multiple logistic regression models were used to examine the adjusted associations between each of the knee OA outcomes at follow-up and CCL2 at baseline (log-scale and tertiles, respectively), both unadjusted as well as adjusted for continuous age and BMI, and categorical race and sex. Trend tests were conducted to determine any increasing or decreasing linear effect on the outcomes across the CCL2 tertiles. To examine whether there was significant heterogeneity of effect, pairwise interactions between CCL2 and each of the covariates were considered. Further, to examine whether there was significant evidence of non-linearity of continuous CCL2, polynomials (i.e., quadratic and cubic term) were fitted. Regression coefficients for these terms were tested and dropped from the model if found to be non-significant.

A power calculation was performed based on the knee-rOA outcome. Using baseline CCL2 tertiles, a sample size of 56 per group would provide 81% power to detect an odds ratio of 3.0 in pairwise comparisons, assuming a proportion of 0.36 in the reference group (i.e., the observed proportion with knee-rOA in the lowest tertile) and a two-sided 0.05 significance level.

The study is viewed as descriptive; accordingly, no adjustment for multiple comparisons was made. The two-sided 0.05 significance level is used, and statistical analyses were performed using SAS software 9.4 (Cary, NC).

RESULTS

Subject characteristics are shown in Table-1. Participants had a median age of 57 years, median BMI of 29 kg/m^2, were 63% women, and 58% Caucasian. The median baseline CCL2 was 292pg/ml. Knee-rOA was present in 45% of the sample at follow-up with 51% showing progression of knee-rOA (Table-1), which is comparable to what was previously reported for the JoCoOA cohort overall[13]. Knee-SxOA was present in 36% of the participants at follow-up. No statistically significant differences were found with respect to these variables between participants with and without missing values.

The percentages of participants with knee-rOA at follow-up increased across the tertiles of baseline CCL2 levels (36.4%, 44.4%, and 53.6%, respectively). An increasing trend was also observed for progression of knee-rOA across the CCL2 tertiles (40.4%, 49.1%, and 61.8%, respectively).

Table-2 presents both unadjusted and adjusted odds ratios, with corresponding 95% confidence intervals (CI) from the logistic models, as well as results for the linear trend testing. In adjusted logistic models, continuous log CCL2 was significantly associated with knee-rOA: for each unit increase in log CCL2, the odds of having knee-rOA at follow-up was increased by 72%. Tertiles of CCL2 showed significant increasing linear trends for knee-rOA, progression of knee-rOA, medial JSN, and progression of medial JSN. To illustrate the increasing trend, the point estimates for the odds of having knee-rOA at follow-up in participants with CCL2 baseline levels in the second and third tertiles were more than twice and three times, respectively, as high as those participants in the lowest tertile of CCL2. Likewise, the odds ratios for progression of knee-rOA from baseline to follow-up in participants with CCL2 levels in the second and third tertiles were increased.

No significant associations were found for knee osteophytes or knee osteophyte progression. We found significant associations for knee JSN, where the odds of having knee JSN in participants in the highest CCL2 tertile were almost three times as high as the odds in participants in the lowest tertile. JSN progression from baseline to follow-up showed a similar pattern. No statistically significant associations were seen for Knee-SxOA, measured with either the binary (Table 2) or the 3-level variable (data not shown) assessment of pain, or for symptoms measured with the WOMAC pain sub-scale (data not shown). Finally, no significant interactions nor polynomial terms were found.

DISCUSSION

Our study reports for the first time an association between serum CCL2 levels and knee-rOA. Specifically, participants exhibiting CCL2 concentrations in the second and third tertiles at baseline were more likely to have higher K/L grades of knee-rOA at follow-up than participants with CCL2 concentrations in the lowest tertile; such associations were strongest for the comparisons with the highest tertile.

We also found a significant association across CCL2 tertiles for medial JSN or JSN progression. JSN is one of the primary knee OA radiographic outcomes, used as a proxy for cartilage loss and soft tissue changes, some of the main correlates of symptoms. However, in spite of CCL2 association with JSN, we found no significant associations between CCL2 and SxOA. Many factors may be related to knee OA pain, such as synovitis, knee effusions, popliteal cysts, bone marrow lesions, meniscal tears, co-medications, as well as psychological and social determinants[14]. Our definition of SxOA used radiographic OA as the structural OA criterion and either 2- or 3-level variables for pain assessment; however, it is possible that a definition of SxOA using other factors, other pain scales, or repeated assessments of symptoms, might yield a different result.

We found no statistically significant association across CCL2 tertiles for osteophyte formation or bone sclerosis (data not shown), suggesting a differential CCL2 action on osteoblasts and osteoclasts/macrophages. Microarray data showing CCR2 up-regulation in OA bone tissues[15], suggest a role of CCR2 in OA bone damage. Using pre-clinical models, we demonstrated that CCR2 mediates bone damage during early-moderate OA, but not at severe stages, when inflammation increases. Therefore, the bone damage may reflect an opposite action of CCL2 on different target cells, (osteoblasts or osteoclasts/macrophages) at different disease stages and might be dependent on the inflammation status.

This longitudinal study has several strengths. First, the sampling strategy includes participants with different racial backgrounds, sex, and a wide range of ages, BMIs, and OA status. We evaluated multiple knee OA outcomes, including individual radiographic features and several ways of defining knee symptoms. This study was limited to the analysis of a single chemokine at a single baseline time point. Additional studies examining change in chemokine levels, simultaneous analysis of other chemokines/cytokines together with additional variables, imaging modalities sensitive to soft tissue changes (Magnetic Resonance or ultrasound), could potentially yield more impactful results. Further, repeated assessments of symptoms using more sensitive or joint-specific pain measures (joint-specific WOMAC) might be more revealing. Finally, we do not know how a CCL2 serum level relates to synovial CCL2 at the affected joint.

In general, a serum biomarker assessment potentially represents an ideal minimally-invasive method for diagnostic/prognostic purposes. Serum CCL2 is unlikely to be sufficient alone for these purposes, but higher levels may be potentially useful to elucidate mechanisms of joint damage and identify individuals at higher odds of structural joint changes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Characteristics of the study population (N=168)

	Variables*	*Frequency or median*	*% or (min, max)*
Baseline	**Age (years)**	57	(45, 78)
	BMI (kg/m^2)	28.9	(15.9, 54.2)
	CCL2 at baseline (pg/ml)	291.71	(21.04, 2192.27)
	Women	105	62.5
	Caucasian	98	58.3
Follow-up	**Knee rOA**	74	44.9
	Knee Sx	91	54.2
	Knee Sx Severity		
	None	77	45.8
	Mild	40	23.8
	Moderate/Severe	51	30.4
	WOMAC pain score		
	>=3	93	55.7
	<3	75	44.3
	Knee SxOA**	59	35.8
	Knee rOA progression	81	50.6
	Knee Osteophytes Medial (Tibial, Femoral)	92	63.5
	Knee Osteophytes progression Medial (Tibial, Femoral)	54	37.5
	Knee JSN Medial	72	50.0
	Knee JSN progression Medial	53	37.1

* Some variables have missing values: Knee rOA, N=165; Knee Osteophytes, N=145; Knee JSN, N=144.

** Knee SxOA requires at least one knee to have evidence of both knee rOA and knee Sx (symptoms). Knee K/L grades at the follow-up: K/L=0 (n=48, 29.1%), K/L=1 (n=43, 26.0%), K/L=2 (n=27, 16.4%), K/L=3 (n=28, 17.0%), K/L=4 (n=19, 11.5%).

Table 2

Unadjusted odds ratios (OR), adjusted odds ratios, and 95% confidence intervals (CI) for associations between CCL2 at baseline and knee OA outcomes at follow-up

Knee OA Outcomes	CCL2 (n /% #)	Unadjusted OR (95% CI)	Adjusted OR* (95% CI)
Knee rOA	Log (CCL2)	1.52 (0.94, 2.47)	**1.72 (1.04, 2.83)**
	Tertile 1 (20/36.4)	reference	reference
	Tertile 2 (24/44.4)	1.42 (0.70, 2.89)	2.18 (0.99, 4.79)
	Tertile 3 (30/53.6)	**2.37 ** (1.17,4.80)**	**3.15 ** (1.38, 7.19)**
Knee rOA Progression	Log (CCL2)	1.40 (0.89, 2.19)	1.40 (0.86, 2.28)
	Tertile 1 (21/40.4)	reference	reference
	Tertile 2 (26/49.1)	1.28 (0.63, 2.58)	1.65 (0.77, 3.57)
	Tertile 3 (34/61.8)	**2.05 ** (1.04, 4.06)**	**2.23 ** (1.03, 4.87)**
Knee Osteophytes Medial	Log (CCL2)	0.87 (0.55, 1.37)	0.89 (0.54, 1.48)
	Tertile 1 (29/65.9)	reference	reference
	Tertile 2 (28/56.0)	0.76 (0.36, 1.62)	1.11 (0.45, 2.75)
	Tertile 3 (35/68.6)	1.09 (0.52, 2.29)	1.30 (0.53, 3.18)
Knee Osteophytes Progression Medial	Log (CCL2)	1.06 (0.67, 1.68)	1.11 (0.65, 1.89)
	Tertile 1 (13/30.2)	reference	reference
	Tertile 2 (20/40.0)	1.20 (0.54, 2.67)	1.72 (0.67, 4.40)
	Tertile 3 (21/41.2)	1.50 (0.67, 3.37)	1.84 (0.69, 4.86)
Knee JSN Medial	Log (CCL2)	1.52 (0.95, 2.41)	1.54 (0.93, 2.54)
	Tertile 1 (18/40.9)	reference	reference
	Tertile 2 (24/48.0)	1.39 (0.67, 2.88)	1.70 (0.78, 3.70)
	Tertile 3 (30/60.0)	**2.60 ** (1.24, 5.43)**	**2.83 ** (1.26, 6.35)**
Knee JSN Progression Medial	Log (CCL2)	1.37 (0.85, 2.22)	1.38 (0.83, 2.27)
	Tertile 1 (12/27.9)	reference	reference
	Tertile 2 (17/34.0)	1.33 (0.58, 3.09)	1.85 (0.78, 4.40)
	Tertile 3 (24/48.0)	2.15 (0.97, 4.78)	**2.43 ** (1.05, 5.62)**
Knee SxOA	Log (CCL2)	1.13 (0.68, 1.88)	1.09 (0.62, 1.92)
	Tertile 1 (17/30.9)	reference	reference
	Tertile 2 (19/35.2)	1.21 (0.55, 2.70)	1.47 (0.61, 3.56)
	Tertile 3 (23/41.1)	1.56 (0.71, 3.40)	1.57 (0.64, 3.85)

* Adjusted ORs from logistic models adjusted for continuous age and BMI, and categorical race and gender.

[#] Frequency and percentage of each CCL2 tertile for each knee OA outcome. CCL2 range in each tertile: tertile 1 (21.04, 221.30), tertile 2 (223.54, 358.10), tertile 3 (373.82, 2192.27) (Supplementary Fig S1)

[**] Statistically significant linear trend tests showing increasing effect on the outcome across the tertiles of CCL2.