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## Serum transforming growth factor-beta 1 is not a robust biomarker of incident and progressive radiographic osteoarthritis at the hip and knee: The Johnston County Osteoarthritis Project

Amanda E. Nelson<sup>1</sup>, Yvonne M. Golightly<sup>1</sup>, Virginia B. Kraus<sup>2</sup>, Thomas Stabler<sup>2</sup>, Jordan B. Renner<sup>1,3</sup>, Charles G. Helmick<sup>4,\*</sup>, and Joanne M. Jordan<sup>1</sup><sup>1</sup>Thurston Arthritis Research Center, University of North Carolina School of Medicine, Chapel Hill, NC<sup>2</sup>Duke University Medical Center, Durham, NC<sup>3</sup>University of North Carolina, Department of Radiology, Chapel Hill, NC<sup>4</sup>Centers for Disease Control and Prevention, Atlanta, GA

### Abstract

**Purpose:** To test whether serum transforming growth factor-beta 1 (TGF- $\beta$ 1) predicts incident and progressive hip or knee radiographic OA (rOA).**Methods:** Serum TGF- $\beta$ 1 was measured for 330 participants aged 45 years and older in the Johnston County Osteoarthritis Project, with paired longitudinal films available for 618 hips and 658 knees. Incident and progressive rOA were defined using Kellgren-Lawrence (K-L) grade as well as osteophyte (OST) and joint space narrowing scores (JSN). Natural logarithm transformation was used to produce near-normal distributions for continuous TGF- $\beta$ 1 (lnTGF- $\beta$ 1). Separate multivariable Weibull regression models were used to provide hazard ratios (HR) for a 1-unit increase lnTGF- $\beta$ 1 with each rOA outcome, accounting for variable follow-up times and clustering by individual, adjusted for age, race, gender, and body mass index (BMI). Interaction terms were considered statistically significant at  $p < 0.10$ .**Results:** The mean ( $\pm$ SD) age of the sample was  $61.9 \pm 9.7$  years, the mean BMI was  $30.3 \pm 6.9$  kg/m<sup>2</sup>, with 60.6% women and 42.4% AA. The mean ( $\pm$ SD) TGF- $\beta$ 1 was  $17.8 \pm 6.1$  ng/ml; follow

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**Corresponding Author:** Amanda E. Nelson, M.D. Thurston Arthritis Research Center, University of North Carolina School of Medicine 3300 Thurston Building, Campus Box #7280 Chapel Hill, NC 27599-7280 Phone (919)966-0553, FAX (919)966-1739 aenelson@med.unc.edu.**Author information:** A.E. Nelson, MD, Assistant Professor, Department of Medicine, University of North Carolina School of Medicine Y.M. Golightly, PT, MS, Doctoral Student, Department of Epidemiology, University of North Carolina Gillings School of Global Public Health

V.B. Kraus, MD, PhD, Associate Professor, Department of Medicine, Duke University Medical Center

T. Stabler, MS, Research Analyst II, Department of Medicine, Duke University Medical Center

J.B. Renner, MD, Professor, Department of Radiology, University of North Carolina School of Medicine

\*C.G. Helmick, MD, Medical Epidemiologist, Centers for Disease Control and Prevention, Atlanta, GA

J.M. Jordan, MD MPH, Professor, Departments of Medicine and Orthopaedics, University of North Carolina School of Medicine

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up time was  $6.1 \pm 1.3$  years. There were no significant interactions by race or gender. HRs showed no significant relationship between lnTGF- $\beta$ 1 and incident or progressive rOA, OST, or JSN, at the knee or the hip.

**Conclusions:** Levels of TGF- $\beta$ 1 do not predict incident or progressive rOA, OST, or JSN at the hip or knee in this longitudinal, population-based study, making it unlikely that TGF- $\beta$ 1 will be a robust biomarker for rOA in future studies.

## Keywords

Osteoarthritis; Biomarkers; Transforming growth factor-beta 1 (TGF- $\beta$ 1); Radiography

## Introduction

There are as yet no proven effective disease-modifying agents for OA, one of the most common causes of disability in the U.S.(1). One of the main reasons for the lack of effective interventions is the lack of an accepted “gold standard” measure of incident and progressive OA. Semiquantitative joint space narrowing and global measures such as the Kellgren-Lawrence (K-L) grade from conventional radiographs are widely used to determine OA progression at the knee or the hip, but can be insensitive to change over time (2;3). Due to the lack of highly sensitive radiographic outcome measures, studies, especially clinical trials, using these outcomes must have follow-up times on the order of years, making such studies prohibitively time-consuming and expensive. Magnetic resonance imaging may in the future replace conventional radiographs as the modality of choice for imaging in knee OA (4;5), but is currently plagued by lack of availability, lack of accepted and widely validated scoring systems, long examination and interpretation times, and cost, and little has been published on MRI in hip OA (6). There is a need for better markers of disease incidence and progression in osteoarthritis (OA). As such, biochemical markers of joint metabolism are appealing, as they are readily available, requiring only blood or urine collection, repeatable, and relatively inexpensive.

Transforming growth factor-beta 1 (TGF-EP $\beta$ 1) is a multifunctional growth factor with an important role in cartilage matrix metabolism, making it an attractive potential biomarker of OA. A few studies examining serum TGF- $\beta$ 1 as a potential biomarker of OA have shown varying results (7-10). The TGF- $\beta$  family consists of three closely related isoforms, TGF- $\beta$ 1, - $\beta$ 2, and - $\beta$ 3, with TGF- $\beta$ 1 being the most abundant and most often studied in relation to bone and OA (11). Compared to controls, patients with OA have elevated TGF- $\beta$ 1 in synovium (12), elevated TGF- $\beta$ 1 expression in mesenchymal stem cells (13), and elevated expression of all three TGF- $\beta$  isoforms in cartilage (14). Exogenous administration of TGF- $\beta$ 1 into murine knee joints leads to increased proteoglycan content in superficial regions of articular cartilage, focal proteoglycan loss in deeper areas, and chondrocyte/osteocyte formation similar to that seen in human OA (15;16). Inhibition of endogenous TGF- $\beta$  (all isoforms), while dramatically decreasing osteophyte (OST) size, also significantly increases proteoglycan loss (17). In rabbits, levels of TGF- $\beta$ 1, shortly after meniscectomy, reflect longer-term bone and cartilage changes (18), suggesting that this marker has the potential to predict OA incidence and progression.

We first became interested in TGF- $\beta$ 1 as a biomarker of radiographic OA (rOA) given the increased osteophyte formation, higher levels of TGFC role of TGF- $\beta$ 1 among African Americans compared to Caucasians (8;19), and our observation of higher osteophyte burden among African Americans compared to Caucasians (20;21). We recently reported the results of a cross-sectional analysis of TGF- $\beta$ 1 and rOA, which did not demonstrate an association with the presence or severity of hip or knee OA, although there was a borderline significant association

between prevalent OST and OST severity that was seen only in African Americans and not in Caucasians (8). Considering the anabolic role of TGF- $\beta$  in osteophyte formation, which is often an early finding in OA, we hypothesized that TGF- $\beta$  may be an important biomarker for incident OA. The current analysis was designed to assess the relationship between baseline TGF- $\beta$ 1 levels and the incidence and progression of rOA, separately for hips and knees, overall and stratified by race, using both Kellgren-Lawrence grade and individual radiographic features (OST and joint space narrowing [JSN]).

## Patients and Methods

Baseline data from 330 participants aged 45 years and older in the Johnston County Osteoarthritis Project were used in the analysis. These patients were seen for a baseline visit (1991-1997) and a follow up visit (1999-2003), approximately 5 years later. Participants for baseline biomarker assessment were selected using a simple random sampling procedure to represent approximately equal proportions of African Americans, Caucasians, women, men, and those with and without knee rOA, with a representative range of ages; for the current analysis, we required complete radiographic data at baseline and a follow up visit, resulting in the inclusion of 330 individuals. Subject recruitment and overall project design procedures have been described for the parent study (22). Demographic and clinical information was obtained by interview, including self-reported race. Participants underwent a clinical evaluation which included measurement of height (m) without shoes and weight (kg) measured with a balance beam scale, followed by calculation of body mass index (BMI, in kg/m<sup>2</sup>). All included participants underwent standardized bilateral weight-bearing anteroposterior radiography of the knees in extension using foot mat positioning with a horizontal x-ray beam centered at the level of the superior patellae. Standardized supine anteroposterior pelvic radiographs were taken in 10-15 degrees of internal rotation with the x-ray beam centered approximately 2.5 cm superior to the pubic symphysis. Women under 50 years of age did not undergo pelvic radiography and were excluded from analyses of hip rOA. Radiographs were read paired without knowledge of participant clinical status or chronological order of the films by a single radiologist (JBR) using the K-L radiographic atlas (23); inter- and intra-rater reliability for this reader are high ( $\kappa=0.86$  and  $0.89$ , respectively) (24).

Incident rOA at the hip or knee was defined in two ways: “Incident rOA 1” (development of K-L grade  $\geq 1$  from a baseline K-L grade = 0), and “Incident rOA 2” (development of K-L grade  $\geq 2$  from a baseline K-L grade  $< 2$ ). Progression of rOA was similarly defined: “Progressive rOA 1” was an increase by  $\geq 1$  K-L grade from a baseline K-L grade  $\geq 1$ , while “Progressive rOA 2” was an increase of  $\geq 1$  K-L grade from a baseline K-L grade  $\geq 2$ . Additionally, to estimate effects on OA in multiple joints (hips or knees), the development of KL  $\geq 2$  in an *additional* joint, in individuals with a baseline K-L grade  $\geq 2$  in at least one joint, was considered as a separate outcome. Incident OST and JSN were defined as joints with grade = 0 at baseline and  $\geq 1$  at follow-up, based on the Burnett atlas (25); progression was defined as an increase of  $\geq 1$  grade from a baseline grade  $\geq 1$ .

After collection at the baseline clinic visit, blood and sera were separated and stored on ice. Sera were frozen within 8 hours of collection at  $-20^{\circ}\text{C}$ , then transferred for long-term storage to a  $-86^{\circ}\text{C}$  environment. The samples were analyzed at Duke University Medical Center (by TS), where TGF- $\beta$ 1 was measured using a sandwich ELISA kit from Biosource International (Camarillo, CA). This method includes an extraction step to release TGF- $\beta$ 1 from latent complexes, thus measuring total serum concentrations. Manufacturer reported precision for this kit was 5.5-6.2% intra-assay CV, and 6.6-7.9% inter-assay CV. Actual inter-assay CV for the assays performed for this study was  $< 6.2\%$ .

The natural logarithm transformation was used to produce near-normal distributions for TGF- $\beta$  (lnTGF- $\beta$ 1) in analyses. Descriptive statistics were calculated for race, gender, age, body mass index (BMI), and lnTGF- $\beta$ 1. Separate multivariable Weibull regression models were used to provide hazard ratios for a 1-unit increase lnTGF- $\beta$ 1 with each rOA outcome, accounting for variable follow-up times and clustering of joints by individual. Cox proportional hazard models, estimating the time of each OA outcome as the midpoint of the follow-up period, were run for comparison with Weibull models and showed similar results; the results of the Weibull models are presented. Models were adjusted for race and gender as categorical variables, and for age and BMI as continuous variables. Interaction terms were considered statistically significant at  $p < 0.10$ . Due to prior results suggesting stronger associations among African Americans, stratification by race was also planned, regardless of interaction results, as an exploratory analysis. Analyses were performed using SAS version 9.1 (Cary, NC) with Stata 10 (College Station, TX) for the Weibull models.

## Results

Three hundred thirty individuals had TGF- $\beta$ 1 measurements; of these, 329 had paired knee films and 309 had paired hip films available for longitudinal analysis. The mean ( $\pm$ SD) age of the overall sample was  $61.9 \pm 9.7$  years, with a mean BMI of  $30.3 \pm 6.9$  kg/m<sup>2</sup> (Table 1). Of the participants overall, 61% were women and 42% were African American. Because women less than 50 years of age did not undergo hip radiography and were therefore excluded from hip-based analyses, there were fewer women with paired hip films, and that group was slightly older than the overall sample (Table 1). The mean ( $\pm$  SD) serum TGF- $\beta$ 1 level was  $17.8 \pm 6.1$  ng/ml (range 6.1 – 40.9 ng/ml). Follow-up time ranged from 3 to 9.8 years, with a mean ( $\pm$  SD) of  $6.1 \pm 1.3$  years. There were no statistically significant interactions by race or gender. Differences between the values of hazard ratio (HR) point estimates and 95% confidence intervals were minimal before and after adjustment; the results adjusted for age, gender, race, and BMI are presented.

### Incident rOA

As shown in Table 2, there were no significant associations between lnTGF- $\beta$ 1 and incident rOA at the hip or the knee. All of the confidence intervals include 1.0, and therefore show no significant alteration in HRs for rOA associated with lnTGF- $\beta$ 1 levels. Similarly, baseline lnTGF- $\beta$ 1 levels did not predict incident JSN at the hip or at the knee, and although there was a 40% increase in the HR for incident OST at both the hip and knee, this did not reach statistical significance (Table 2). The HRs were also not significant after stratification by race (Incident OST at the knee for African Americans  $n=33$ , HR 0.50, 95% CI 0.12-2.08; for Caucasians  $n=61$ , HR 2.06, 95% CI 0.66-6.48).

### Progressive rOA

Table 3 shows the adjusted HRs for progressive rOA at the hip or knee, again without significant association between HRs for progressive rOA and lnTGF- $\beta$ 1 levels. There were small numbers for progressive rOA at the hip, especially for progression from a baseline K-L grade  $\geq 2$  (progressive rOA 2), and for progressive JSN. The risk of progressive knee rOA by either K-L definition was 40-50% higher in association with higher lnTGF- $\beta$ 1 levels, and there was an apparent 45% reduction in risk for progressive OST at the hip and the knee, but these relationships did not reach statistical significance (Table 3). Stratification by race did not identify significant associations, but there was again a suggestion of reduction in HR among African Americans for progressive OST at the knee ( $n=62$ ; HR 0.39, 95% CI 0.15-1.03) compared to Caucasians ( $n=14$ , HR 2.14, 95% CI 0.36-12.7). The HR for progressive JSN at

the knee was not significantly increased for either African Americans (n=59, HR 1.64, 95% CI 0.66-4.09) or Caucasians (n=25, HR 1.10, 95% CI 0.26-4.70).

We also considered individuals (n=57) who had a K-L grade of  $\geq 2$  at least one joint at baseline, and then developed K-L grade  $\geq 2$  at an additional hip or knee that was not involved at baseline. The adjusted HR for this outcome was 1.08 (95% CI: 0.45-2.61), again indicating no significant association between lnTGF- $\beta$ 1 levels and development or progression of OA.

## Discussion

In this analysis, levels of TGF- $\beta$ 1 did not predict development of incident rOA, OST, or JSN in the hip or knee. The small increase in progressive knee rOA risk with higher TGF- $\beta$ 1 was not statistically significant, despite a large proportion of the sample having these outcomes. There was no association between TGF- $\beta$ 1 levels and progression of rOA in additional joint sites (across both knees and both hips). The small, non-significant alterations in risk identified in the current study, despite a large overall sample size and fairly large proportion with most of the outcomes of interest, demonstrate that serum TGF- $\beta$ 1 is not likely to be a robust biomarker of rOA. This is in agreement with our findings in a cross-sectional study looking at prevalence and severity of radiographic OA, in which no significant associations were seen with levels of serum TGF- $\beta$ 1(8).

Despite our prior findings of greater OST burden in African Americans (20;21) and the trend toward higher TGF- $\beta$ 1 with more severe OST grades (8), we did not see any interactions by race in the current analysis, and stratified analysis did not reveal significant differences. We had limited power to detect interactions and differences by race due to a small number of outcomes by race in stratified analyses. There was a suggestion of reduction in risk of both incident and progressive OST at the knee with higher baseline serum TGF- $\beta$ 1 levels among African Americans. However, the differences were not statistically significant, and there were small numbers for some of the outcomes following stratification, so these findings must be considered very cautiously. One could speculate that there may be higher levels of synovial TGF- $\beta$ 1 acting locally leading to these findings, but we did not have synovial fluid levels to evaluate this.

Prior studies of TGF- $\beta$ 1 in OA have failed to show consistent results. Otterness and colleagues assessed a panel of biomarkers in a case control study with 12 month follow up and biomarker measurement at multiple time points. In agreement with our cross-sectional study of the current cohort (8), they found that levels of TGF- $\beta$ 1 did not discriminate between individuals with OA (by ACR criteria) and controls (7). They did identify a borderline association between baseline TGF- $\beta$ 1 levels and change in clinical status (as determined by change in symptoms and patient/physician assessments) at 12 months, primarily because of an association between patient global assessment and baseline TGF- $\beta$ 1 (10). Another group, using radiographic data in addition to clinical criteria, showed a small, negative correlation ( $r = -0.19$ ,  $p$  value 0.01) between baseline TGF- $\beta$ 1 level and K-L grade at the knee, but in contrast to the Otterness study, no associations were seen with clinical outcomes at baseline or 18 month follow-up (9). These studies, in combination with the negative results of the current study, suggest that serum TGF- $\beta$ 1 is unlikely to be a useful biomarker for future study in OA.

Limitations to this analysis include the lack of symptoms data, and the small number of hips demonstrating progression, especially for those progressing beyond K-L grade 2 and with progressive JSN. We had only a single measurement of TGF- $\beta$ 1 at baseline rather than repeated measures at multiple timepoints. We had radiographic data only for the hips and knees, and therefore cannot account for OA at other joint sites. As in most large epidemiologic studies (26), the hip radiographs in this study are non-weightbearing, and there remains controversy

in the literature regarding the benefits of weightbearing versus non-weightbearing standardized hip radiographs (27;28). Also, and primarily for logistic reasons, because of the size and complexity of the parent study, it was not possible to control for diurnal variation, exercise levels, postural changes, or dietary intake that may alter serum TGF- $\beta$ 1 levels (29). A study of diurnal variation in OA biomarkers by Kong and colleagues demonstrated a correlation between TGF- $\beta$ 1 levels and summed K-L grade for both knees, but only at the end of the day ( $r^2=0.35$ ,  $p=0.006$ ), with no correlation between K-L grade and morning TGF- $\beta$ 1 levels, before or after food intake or activity (30). The local action of TGF- $\beta$ 1 in the joint, as suggested by animal studies of exogenous supplementation and endogenous inhibition of TGF- $\beta$ 1 (15;17), suggest the potential usefulness of this marker as measured in synovial fluid. However, as the need for biomarkers is primarily to facilitate large, longitudinal studies and clinical trials of therapeutics, evening collections and invasive procedures such as arthrocentesis, even if stronger associations were seen, would make this marker impractical.

The strengths of our study are the inclusion of African American and Caucasian men and women and the collection of longitudinal radiographic data at two large joint sites over a mean of 6 years. This is the largest longitudinal study to date of TGF- $\beta$ 1 as a biomarker of rOA. Although we have observed higher levels of TGF- $\beta$ 1 among African American individuals, there is no race interaction for the relationship of rOA and TGF- $\beta$ 1, indicating no significant difference by race in the effect of this growth factor. Despite the large size of our sample and the use of a joint-based analysis to maximize sample size for this longitudinal analysis, we did not identify significant associations between serum TGF- $\beta$ 1 levels and incident or progressive rOA at the hip or knee. This finding, in combination with the lack of association identified in our cross-sectional analysis, indicates that serum TGF- $\beta$ 1 is unlikely to be a robust biomarker of OA for future studies.

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**Table 1**

Sample characteristics at the baseline visit\*

Characteristic	Total sample (n=330)	Paired Knee films (n=329)	Paired Hip films (n=309)
Age (years)	61.9 (9.7)	61.9 (9.7)	62.3 (9.3)
BMI (kg/m <sup>2</sup> )	30.3 (6.9)	30.3 (6.9)	30.2 (6.7)
Female (%)	60.6	60.8	59.2
African American (%)	42.4	42.6	40.8

\* Given as mean (SD) or percentage

**Table 2**Adjusted hazard ratios for serum TGF- $\beta$ 1 and incident radiographic OA (rOA)

<b>Incident rOA Outcome</b>	<i>n (hips with outcome)</i>	<b>Adjusted* HR<sup>†</sup> for Hip rOA (95% CI)</b>	<i>n (knees with outcome)</i>	<b>Adjusted* HR<sup>†</sup> for Knee rOA (95% CI)</b>
<b>Incident rOA 1</b> <i>from baseline K-L&lt;1 to K-L<math>\geq</math>1</i>	54	1.05 (0.31-3.51)	94	1.04 (0.41-2.65)
<b>Incident rOA 2</b> <i>from baseline K-L&lt;2 to K-L<math>\geq</math>2</i>	45	0.61 (0.25-1.47)	103	1.10 (0.46-2.63)
<b>Incident OST</b> <i>from grade 0 to grade <math>\geq</math>1</i>	49	1.41 (0.38-5.25)	94	1.41 (0.56-3.56)
<b>Incident JSN</b> <i>from grade 0 to grade <math>\geq</math>1</i>	29	0.65 (0.20-2.09)	99	1.39 (0.50-3.88)

\* Adjusted for age, gender, race, and BMI

<sup>†</sup> Hazard ratio for a 1-unit increase in serum TGF- $\beta$ 1

**Table 3**

Adjusted hazard ratios for progressive radiographic OA (rOA)

<b>Progressive rOA Outcome</b>	<i>n (hips with outcome)</i>	<b>Adjusted*HR<sup>†</sup> for Hip rOA (95% CI)</b>	<i>n (knees with outcome)</i>	<b>Adjusted*HR<sup>†</sup> for Knee rOA (95% CI)</b>
<b>Progressive rOA 1</b> <i>increasing <math>\geq</math> 1 grade from baseline K-L <math>\geq</math> 1</i>	47	1.02 (0.46-2.28)	160	1.51 (0.82-2.79)
<b>Progressive rOA 2</b> <i>increasing <math>\geq</math> 1 grade from baseline K-L <math>\geq</math> 2</i>	10	2.74 (0.31-24.30)	86	1.36 (0.63-2.91)
<b>Progressive OST</b> <i>increasing <math>\geq</math> 1 grade from baseline OST <math>\geq</math> 1</i>	19	0.55 (0.12-2.59)	76	0.55 (0.24-1.26)
<b>Progressive JSN</b> <i>increasing <math>\geq</math> 1 grade from baseline JSN <math>\geq</math> 1</i>	7	4.14 (0.62-27.7)	84	1.40 (0.64-3.03)

\* Adjusted for age, gender, race, and BMI

<sup>†</sup> Hazard ratio for a 1-unit increase in serum TGF- $\beta$ 1