

Trabecular Morphometry by Fractal Signature Analysis Is a Novel Marker of Osteoarthritis Progression

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Objective. To evaluate the effectiveness of using subchondral bone texture observed on a radiograph taken at baseline to predict progression of knee osteoarthritis (OA) over a 3-year period.

Methods. A total of 138 participants in the Prediction of Osteoarthritis Progression study were evaluated at baseline and after 3 years. Fractal signature analysis (FSA) of the medial subchondral tibial plateau was performed on fixed flexion radiographs of 248 nonreplaced knees, using a commercially available software tool. OA progression was defined as a change in joint space narrowing (JSN) or osteophyte formation of 1 grade according to a standardized knee atlas. Statistical analysis of fractal signatures was performed using a new model based on correlating the overall shape of a fractal dimension curve with radius.

Results. Fractal signature of the medial tibial plateau at baseline was predictive of medial knee JSN progression (area under the curve [AUC] 0.75, of a receiver operating characteristic curve) but was not predictive of osteophyte formation or progression of

JSN in the lateral compartment. Traditional covariates (age, sex, body mass index, knee pain), general bone mineral content, and joint space width at baseline were no more effective than random variables for predicting OA progression (AUC 0.52–0.58). The predictive model with maximum effectiveness combined fractal signature at baseline, knee alignment, traditional covariates, and bone mineral content (AUC 0.79).

Conclusion. We identified a prognostic marker of OA that is readily extracted from a plain radiograph using FSA. Although the method needs to be validated in a second cohort, our results indicate that the global shape approach to analyzing these data is a potentially efficient means of identifying individuals at risk of knee OA progression.

Osteoarthritis (OA) progression can be defined anatomically via plain radiography, clinically via symptoms, or physiologically via a functional assessment. Of these 3 methods, the anatomic means of assessment is the most common. The only method currently accepted for evaluating disease progression in knee OA is the assessment of joint space narrowing (JSN) using sequential radiographs. Problems with radiographic evaluation of OA include the difficulty of trying to reproduce consistent patient positioning throughout sequential radiographs in order to measure joint space width (JSW) and a relative lack of sensitivity to denote changes in JSW that might otherwise be observed over a longer period of time (18–24 months). Furthermore, changes in JSW are confounded by meniscal damage and extrusion, which are also observed in OA (1). Risk factors such as body mass index (BMI), age, and sex are commonly used in OA clinical trials in an attempt to select individuals at greater risk of knee OA progression (2–4). Unfortunately, the effects and interaction of these predictors are not fully understood, and efforts to use them to predict knee OA progression have not been highly successful.

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The continued lack of a good predictor of OA has stalled the development of treatments of a disease that affects nearly 20% of the population and can have a significant impact on a patient's productivity and quality of life.

Analyses of bone in OA date back more than 50 years and have provided clear indications that changes in periarticular bone occur very early in OA development (5). In the 1990s, Lynch and colleagues were the first to analyze bone architecture on radiographs of OA joints using fractal signature analysis (FSA) (6,7), a technique first used in medicine to study abnormalities observed on lung radiographs (7). FSA evaluates the complexity of detail of a 2-dimensional image (a projection of the 3-dimensional bone architecture) at a variety of scales spanning the typical size range of trabeculae (100–300 μm) and trabecular spaces (200–2,000 μm) (8). As described by Messent et al, the complexity of detail quantified by fractal dimension (FD) is determined principally by the number, spacing, and cross-connectivity of trabeculae (9). Using nuclear magnetic resonance (NMR), it has been determined that the apparent FD is an index of bone marrow space pore size; pore size, in turn, is related to and increases with perforation and disappearance of trabeculae (10). To date, FSA has been applied successfully to the study of osteoporosis and arthritis of the spine (11–14), hips (15,16), knees both before and after joint replacement (6,7,9,14,17–26), knees in which the anterior cruciate ligament (ACL) has ruptured (27), wrists (14,28,29), and hands (14). Plain radiographs have been the image type primarily used in FSA, but FSA can also be used with other image types, such as those acquired using computed tomography (11,12) and NMR (10).

One of the major advantages of using FSA is that many of the pitfalls inherent in evaluating JSN, the gold standard for determining radiographic progression, can be avoided. Measuring JSN is problematic due to the need for high-quality images (often beyond the general quality of clinical images) obtained using rigid acquisition protocols to extract good quantitative data. In particular, FSA has been shown to be robust against potential problems, such as varying radiographic exposure, changing pixel size, and knee repositioning (6). The feasibility of using FSA in a clinical trial was demonstrated retrospectively using the radiographs from a 2-year longitudinal study of bisphosphonate for OA (25). In the current study, we have focused on using FSA to predict progression of knee OA.

Analyses of bone are pertinent to understanding the OA disease process. Bone density measurements have revealed that subchondral and subarticular bone in

subregions of the diseased compartment of OA knees is osteoporotic (30,31). The process of periarticular bone remodeling of OA joints is believed to account for the fact that bone resorption biomarkers have been found to be elevated in patients with progressive knee OA (32), which is consistent with the findings of biomechanical studies demonstrating that the subchondral bone from OA knees is less stiff and dense, is more porous, and has reduced mineral content (33). These findings correspond to results reported by Buckland-Wright, whose FSA study of OA knees revealed both decreased FDs, which can be interpreted as an increase in the thickness of medium-to-large horizontal trabecular structures in early OA, and increased FDs, which can be interpreted as an increase in the fenestration and thinning (and, thus, the total number) of vertical trabecular structures of most sizes in severe OA (severity defined by JSW) (34).

To date, 3 longitudinal studies have used FSA to evaluate changes in tibial cancellous bone in the context of knee OA progression, but results have been conflicting (19,21,25). In the first study, significant differences were noted in the pattern of change over 12 months observed using FSA (increased vertical size of most trabecular structures and decreased horizontal size of large trabeculae) between patients with slow JSN ($n = 240$) and patients with marked JSN ($n = 12$) (19); these results were interpreted as being indicative of local subchondral bone loss coincident with knee OA progression. A second study of a smaller patient population ($n = 40$) failed to identify significant differences in the pattern of change over 24 months observed using FSA between patients with slow OA progression and those whose disease was progressing rapidly (21).

A third study evaluated changes over 3 years observed using FSA in one-third of the patients ($n = 400$) in a placebo-controlled trial of a bisphosphonate for knee OA (25). Compared with patients whose JSN was not progressing, patients with rapidly progressing JSN tended to have a greater decrease in the vertical FDs (interpreted as a greater loss of vertical trabeculae of most sizes), although there was no significant difference observed in horizontal trabeculae. In contrast, the group with JSN that was not progressing exhibited both a slight decrease in FDs for vertical and horizontal trabeculae over time and no drug treatment effect. Those with progressive JSN exhibited a marked and dose-dependent change while receiving drug treatment as observed using FSA, which was consistent with a preservation of trabecular structure, and a reversal of

pathologic changes while receiving increasing drug treatment.

To our knowledge, no prior *in vivo* study has evaluated the utility of FSA for predicting which individuals from a cohort of knee OA patients will have progressive disease. We also sought to undertake the challenge of developing a more holistic method of accounting for the array of variables that are common to such studies. As described herein, we addressed this problem with a generalized “shape analysis” of the data, which enabled us to create an overall model that could predict OA progression independent of other nonradiographic variables.

PATIENTS AND METHODS

Patients. A total of 159 participants (118 women and 41 men) were enrolled in the NIH-sponsored Prediction of Osteoarthritis Progression (POP) study, which was approved by and in accordance with the policies of the Duke University Institutional Review Board. Participants were recruited primarily through rheumatology and orthopedic clinics, and all participants had a diagnosis of OA of at least 1 knee according to the American College of Rheumatology criteria for OA (35). In addition, all participants met radiographic criteria for OA with a score of 1–3 in at least 1 knee according to the Kellgren/Lawrence (K/L) scale (36). Exclusion criteria included the following: a score of 4 in bilateral knees according to the K/L scale; treatment with a corticosteroid (either parenteral or oral) within 3 months prior to evaluation for the POP study; arthroscopic surgery of the knee within 6 months prior to study evaluation; a known history of avascular necrosis, inflammatory arthritis, Paget’s disease, joint infection, periarticular fracture, neuropathic arthropathy, reactive arthritis, or gout involving the knee; and anticoagulation therapy at the time of study evaluation. A total of 186 participants were screened to identify the final 159 participants.

Our analyses were focused on the 138 participants (87%) who returned for followup evaluation 3 years after enrollment in the POP study. Of the 276 knees available for analysis, 10 had been replaced at baseline, and 18 had been replaced during the period of longitudinal followup, leaving a total of 248 knees available for the final analyses. Data on age, sex, and BMI (kg/m^2) were collected as covariates. Symptoms of OA in knees were ascertained using the First National Health and Nutrition Examination Survey criterion of pain, aching, or stiffness (37) on most days of any 1 month in the last year of the study; for patients reporting pain, aching, or stiffness, symptoms were quantified as mild, moderate, or severe, yielding a total score of 0–4 for each knee.

Radiographic imaging. Posteroanterior fixed-flexion knee radiographs were obtained using the SynaFlexer positioning frame (Synarc, San Francisco, CA) with a 10-degree caudal x-ray beam angle (38). Radiographs were scored 0–4 according to the K/L scale, and individual OA radiographic evidence of JSN and osteophyte formation were scored 0–3 according to the Osteoarthritis Research Society International standardized atlas (39) for the medial and lateral tibiofemoral compart-

ments. This resulted in total JSN scores of 0–6 and osteophyte scores of 0–12 (all 4 margins of the knee joint were scored for this feature). Blinded rescoring of 78 knee radiographs was performed to calculate intrarater reliability using the weighted kappa statistic, and results were as follows: for scoring of JSN, $\kappa = 0.71$ (95% confidence interval [95% CI] 0.63–0.79), and for scoring of osteophyte formation, $\kappa = 0.73$ (95% CI 0.67–0.79). For the purpose of statistical modeling, knee OA status at baseline was defined as the JSN score assessed at baseline. Knee OA progression was calculated as the change in JSN score or the change in osteophyte score in the tibiofemoral compartment over 3 years and was derived from baseline and followup radiographs analyzed in tandem by 2 trained readers, who were blinded with regard to clinical data and data regarding bone texture but not to the time sequence.

Of the 248 knees available for analysis, 32 (13%) were defined as having progressive OA on the basis of an increase in JSN over 3 years (18 based on medial JSN, and 14 based on lateral JSN), and 172 (69%) were defined as having progressive OA on the basis of increased osteophyte formation (75 based on medial osteophyte formation, and 97 based on lateral osteophyte formation). It was possible for the knees analyzed to exhibit a change in osteophyte formation while exhibiting no change in JSN. However, except for 1 case, all knees determined to have progressive OA based on increased JSN also had increased osteophyte scores. Trabecular bone mineral density (BMD) and bone mineral content (BMC) were measured at the calcaneus of the dominant leg using an Apollo TM dual x-ray absorptiometry (DXA) bone densitometer (Norland Medical Systems, Fort Atkinson, WI). Knee alignment was measured manually to within 0.5 degrees on a weight-bearing “long-limb” (pelvis to ankle) anteroposterior radiograph as previously reported (40), using the center of the base of the tibial spine as the vertex of the angle.

Image analysis. All radiographs were analyzed using the KneeAnalyzer application (Optasia Medical, Manchester, UK). KneeAnalyzer utilizes computer-aided detection based on statistical shape modeling to provide highly reproducible quantitative measurements of the medial compartment of the knee, yielding separate vertical and horizontal FDs over a range of scales related to trabecular dimensions and referred to as signatures. All radiographs were digitized at 150 dots per inch (which converts to a pixel resolution of 169.3μ) using a Diagnostic Pro Plus digitizer (Vidar, Herndon, VA). According to the requirements of the KneeAnalyzer application, all radiographs were converted from the Digital Imaging and Communication in Medicine (DICOM) format to an uncompressed, 8-bit gray-scale TIFF format using the PixelMed Java DICOM Toolkit (available at www.pixelmed.com). All analyses were performed with the fibula on the left side of the image as viewed by the rater. (Images were turned horizontally as necessary.) Correction for magnification was achieved using KneeAnalyzer, which could detect the vertical column of beads in the SynaFlexer positioning frame.

Joint segmentation was based on the following 6 manually selected initialization points: the lateral femur, the medial femur, the lateral tibia, the medial tibia, the lateral tibial spine, and the medial tibial spine (Figure 1A). Once the initialization points were selected, the software determined the joint space boundary profiles for both the lateral and medial compartments and automatically identified the rectangular

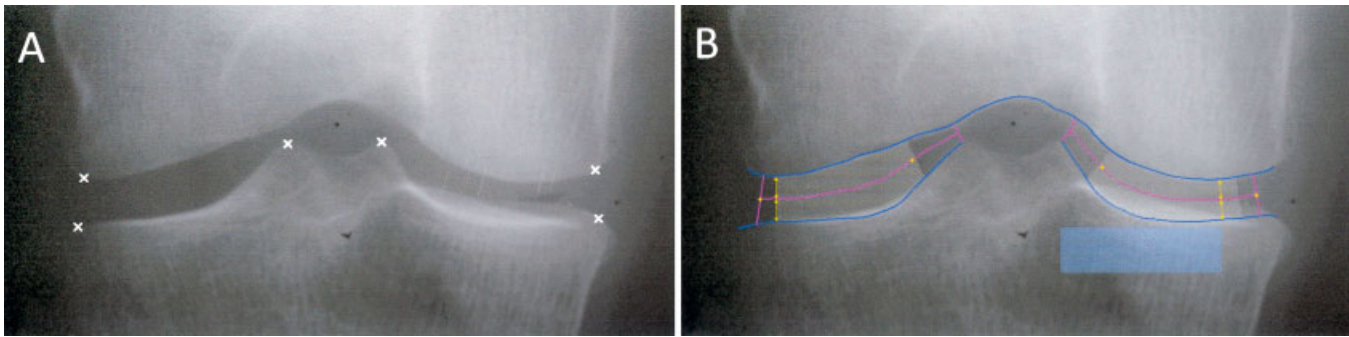


Figure 1. Identification of regions of interest using KneeAnalyzer. **A**, Joint segmentation was based on 6 manually selected initialization points: the lateral femur, the medial femur, the lateral tibia, the medial tibia, the lateral tibial spine, and the medial tibial spine (each marked with an x). **B**, Once the initialization points were selected, KneeAnalyzer was used to determine the joint space boundary profiles for both the lateral compartment and the medial compartment (on the right side of the image) and to identify the region for fractal signature analysis in the medial subchondral bone (blue box).

region in the medial subchondral bone to be used in FSA, based on the medial tibial joint profile (Figure 1B). The FSA region of interest (ROI) spanned three-fourths of the width of the tibial compartment and had a height of 6 mm (determined using SynaFlexer calibration), and a left boundary aligned with the tip of the medial tibial spine. This ROI was standardized based on methods described by Messent et al, who used this area in order to avoid periarticular osteopenia adjacent to marginal osteophytes (9).

From this region, the area to be used in FSA was determined at a range of scales (termed “radii”), based on pixel resolution and SynaFlexer calibration. The radii for the area to be used in FSA ranged in dimension from 3 pixels wide (0.4 mm) to the width of one-half the height of the ROI (3 mm). The FDs in 2 directions were measured with rod-shaped structuring elements (6) using a “box” counting approach (10). FSA data provided by the software are referred to as the “vertical filter” (the horizontal FD) and the “horizontal filter” (the vertical FD). To avoid confusion, we describe the data in terms of the horizontal FD (tension) and the vertical FD (compression), and not according to the filter.

Interrater reliability. A subset of 6 radiographs (3 from OA patients and 3 from non-OA controls) were analyzed by 3 analysts to test whether the FSA evaluation differed among individual analysts. The range and distribution of filter elements and the fractal signature for both the horizontal and vertical FDs were evaluated.

Statistical analysis. Fractal signature data generated by KneeAnalyzer are 3-dimensional, and FDs of compression and tension are measured over a range of radii for each radiograph, representing increasing lengths based on the pixel dimension. The FD measurements are highly correlated along the radius. We modeled the trends of compression and tension change over the radius with second-order (quadratic) multiple regression models using a noncentered polynomial, so that the multidimensional correlations between FD measurements and radii were summarized by 2 polynomial “shape” parameters. Using the shape approach, identical alignment of radii for each patient was not necessary, and all data could be fully used, thereby increasing the power of the study to determine potential differences between groups. Clinical covariates (including

age, sex, BMI, knee pain, BMC, location of the OA [whether the left or right knee], knee alignment, and severity of the OA at baseline) were included in the same statistical model using analyses of covariance and repeated measures. Linear mixed models and generalized linear models were used to adjust for correlations between knees.

To determine if variations in fractal signature were associated with any clinical factors, we tested whether the shapes of polynomial curves were different between different groups of individuals (e.g., patients with progressive OA versus patients with nonprogressive OA). This tested the interaction terms between the shape parameters and the group indicators. We also investigated whether variations in FDs were associated with other clinical factors such as age, sex, BMI, and other covariates, adjusting for the shape of curves considered in the model.

The full statistical model was:

$$Y_{ijk} = u + a + g + \text{BMI} + \text{BMC} + \text{KA} + \text{JSN} + \text{LR} + r_k + r_k^2 + g\text{ID}_i + (r_k \times g\text{ID}_i) + (r_k^2 \times g\text{ID}_i) + P_{ij} + e_{ijk}$$

where Y_{ijk} is the FD reading calculated at i th (status [progressive OA versus nonprogressive OA]), j th (individual location [left knee versus right knee]), and k th (radius); u is the grand mean; a is age; g is gender; KA is knee alignment; JSN is joint space narrowing measured at baseline; LR is the left or right knee indicator; r is radius, linear term; r^2 is radius, quadratic term; $g\text{ID}_i$ is the group ID (e.g., $i = 0$ if OA was progressive, and $i = 1$ if OA was not progressive); $r_k \times g\text{ID}_i$ and $r_k^2 \times g\text{ID}_i$ are the interaction terms; P_{ij} is the random effect associated with the j th subject in group i ; and e_{ijk} is the random error term, associated with the j th subject in group i at radius k . Since FD measures are generally more correlated in radii that are near to one another than in radii that are far apart from one another, a first-order autoregressive (AR) correlation model with mixed/repeated measures (SAS Institute, Cary NC) was used. More sophisticated statistical models were investigated as well, e.g., with various interaction terms between/among fixed effects, and multiple intrasubject random correlation patterns. The model we used was selected because of its parsimony and efficiency.

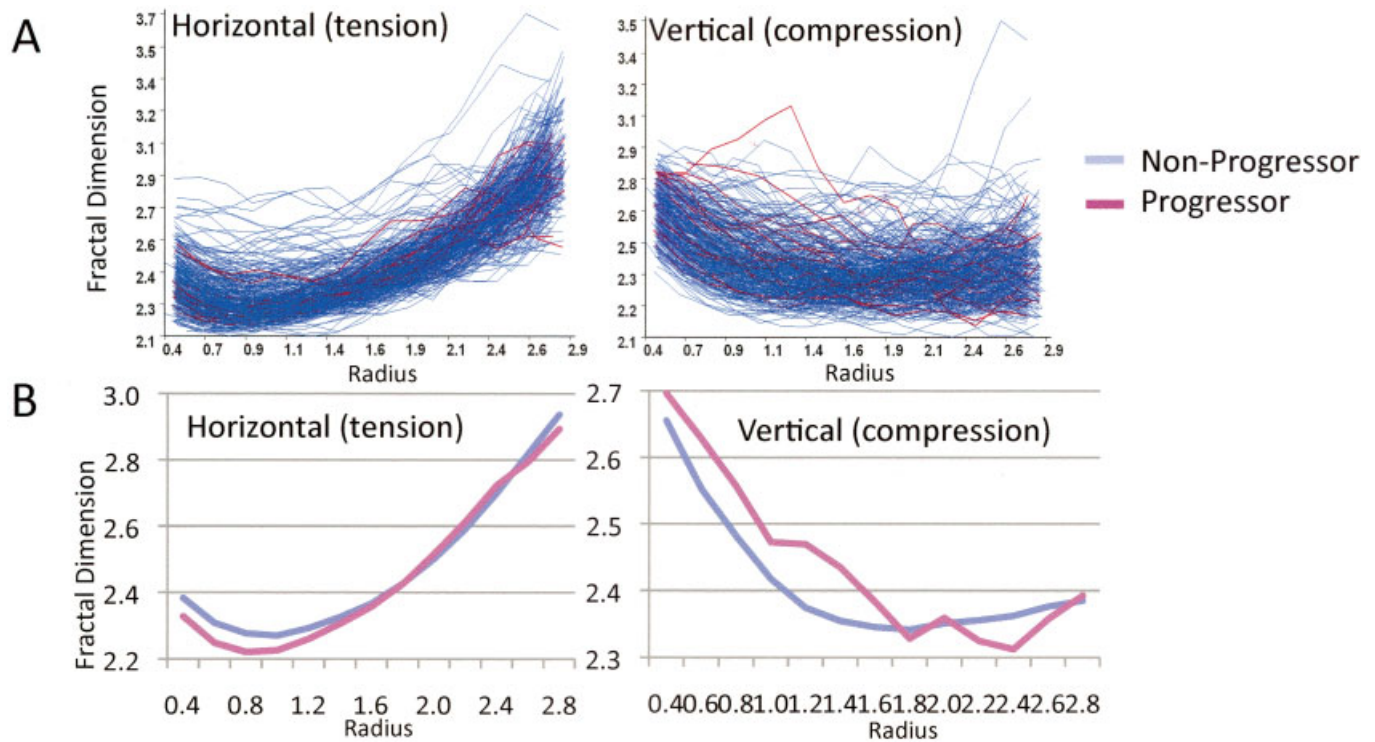


Figure 2. Fractal signature analysis curves from patients with progressive osteoarthritis (OA) and patients with nonprogressive OA. **A,** Analysis of knee radiographs using KneeAnalyzer, which generated a complex family of curves. Each curve represents 1 individual’s fractal signature (reported as fractal dimensions [FDs] over a series of radii [in mm]). **B,** Curve fitting with quadratic and linear components showing lower mean FDs in the tension (horizontal) component and higher mean FDs in the compression (vertical) component, in patients with progressive knee OA.

To determine whether the shapes of the polynomial curves could be used to predict disease progression, we included estimates of the shape parameters of the polynomial curves from both the compression and tension FDs, together with other covariates, in a generalized linear model/generalized estimating equation (GLM/GEE) to predict disease progression status. The GLM/GEE was used to adjust for correlations within an individual because there were 2 curves for most patients (1 for the left knee and 1 for the right knee), and the shape parameters estimated from those curves were likely to be correlated. The linear predictors from the GLM/GEE model were used to predict scores for every knee.

The receiver operating characteristic (ROC) curves were generated based on the prediction scores using cross-validation in 5 folds (or groups), as described by Efron and Tibshirani (41). In the cross-validation, the data were divided randomly into 5 groups; 4 groups were used as training data for model building, and 1 group was used for model validation. False-positive and false-negative rates were calculated by averaging results from all 5 possible training data/validation data combinations. A total of 300 cross-validations were performed, and the averages were reported. Various statistical models containing different combinations of predicting variables were investigated. Data relating to the number of patients that must be screened in order to predict 1 patient with

progressive OA were derived from the ROC curves for a range of Type I error rates.

The full GLM/GEE model was:

$$Y_{ij} = u + a + g + \text{BMI} + \text{BMC} + \text{KA} + \text{JSN} + \text{LR}_j + \text{HL} + \text{HQ} + \text{VL} + \text{VQ} + P_i + e_{ij}$$

where Y_{ij} is the disease progression status (defined as a change in JSN of at least 1 grade or a change in osteophyte formation of at least 1 grade) recorded at i th (individual) and j th (left knee versus right knee); u is the grand mean; a is age; g is gender; KA is knee alignment; JSN is the joint space narrowing at baseline; HL is the linear shape parameter estimated from horizontal filter data; HQ is the quadratic shape parameter estimated from horizontal filter data; VL is the linear shape parameter estimated from vertical filter data; VQ is the quadratic shape parameter estimated from vertical filter data; P is the patient ID (treated as a random effect); and e_{ij} is the random error term, associated with i th subject and j th knee.

RESULTS

Interrater reliability of fractal signatures. The impact of individual analysts on FSA was small and

Table 1. Bivariate associations in patients with progressive and nonprogressive OA*

Factor	Horizontal fractal dimension (tension)		Vertical fractal dimension (compression)	
	Progression based on osteophytes	Progression based on JSN	Progression based on osteophytes	Progression based on JSN
Radius	<0.0001 (-0.426)	<0.0001 (-0.386)	<0.0001 (0.018)	<0.0001 (-0.041)
Radius ²	<0.0001 (0.202)	<0.0001 (0.193)	<0.0001 (0.139)	<0.0001 (0.134)
Left knee/right knee	0.732 (0.011)	0.904 (0.002)	0.148 (0.038)	0.108 (0.050)
Radius + OA progression	0.002 (-0.094)	0.034 (-0.106)	0.969 (0.001)	0.895 (0.006)
Radius ² + OA progression	<0.0001 (0.036)	0.023 (0.034)	0.910 (-0.001)	0.704 (0.005)
Left knee/right knee + OA progression	0.539 (-0.014)	0.994 (0.0003)	0.047 (-0.045)	0.255 (-0.041)
Sex	0.540 (0.009)	0.5729 (0.008)	<0.0001 (-0.075)	<0.0001 (-0.078)
Age	0.323 (-0.0005)	0.3130 (-0.0006)	0.008 (0.001)	0.005 (0.001)
BMI	0.983 (0.00002)	0.916 (0.0001)	0.001 (0.003)	0.0003 (0.003)
Calcaneal BMC	0.0187 (-0.005)	0.018 (-0.005)	0.758 (-0.0007)	0.868 (-0.0004)
Knee pain	0.873 (-0.001)	0.885 (-0.001)	0.004 (0.023)	0.004 (0.023)
Knee alignment	0.673 (0.001)	0.769 (0.0004)	0.292 (0.001)	0.206 (0.002)
JSN status at baseline	0.671 (-0.003)	0.663 (-0.003)	<0.0001 (0.033)	<0.0001 (0.033)

* Values are the *P* value (parameter estimate) determined by analysis of covariance, using a variance component model. Fractal dimension was calculated as the second polynomial fitting over “radius”/progression of osteoarthritis (OA) + clinical covariates + design parameters + random effects (variance components). JSN = joint space narrowing; BMI = body mass index; BMC = bone mineral content.

nonsignificant. In order to test the impact of the analysts, linear regression was used to plot the findings of each analyst versus the mean filter element size or the mean fractal signature (horizontal and vertical) of the 6 knee radiographs. Using horizontal fractal signatures, the intercept and slope (R^2) in the findings of 3 analysts were determined to be 0.105 and 0.958 (0.93), -0.006 and 1.009 (0.86), and -0.99 and 1.032 (0.81). Using vertical fractal signatures, the intercept and slope (R^2) in the findings of 3 analysts were determined to be -0.05 and 1.022 (0.97), -0.13 and 0.94 (0.97), and -0.07 and 1.31 (0.97). Using filter elements, the intercept was determined to be 0 for all 3, and the slope was determined to be 1.002, 1.002, and 0.995 ($R^2 > 0.99$).

Since the box used for FSA is not placed manually, we reviewed both the magnification factor of the Synflexer calibration and the digitally determined location of the box used by the 3 analysts as a possible source of small and nonsignificant variations. In all cases but 1, the magnification factors were identical. In the other case, there was a 2.8% variation between 1 analyst and the other 2. The median box size for the group of patients was 157 pixels (range 140–183) by 39 pixels (range 37–47). The differences in the area of the box were $\leq 9\%$ among the individual analysts and for all patients.

Image analysis. The complexity of the data created an interesting bioinformatic challenge, as demonstrated by the complex curves generated using data from all knees (Figure 2A.) Upon analyzing the total fractal data without global shape analysis (Figure 2A), we found

that there was no statistically significant association between the FDs and the status of disease progression (in horizontal FDs, $P = 0.42$ for progression of osteophyte formation and $P = 0.07$ for progression of JSN; in vertical FDs, $P = 0.67$ for progression of osteophyte formation and $P = 0.15$ for progression of JSN). These results demonstrated the value of analyzing across groups within specific ranges of radii or trabecular size in order to draw meaningful conclusions.

In the past, this was typically done by subtracting the data obtained at baseline from followup FSA data, followed by group comparisons of data within specific ranges of trabecular widths. However, we chose a new method of analysis based on correlating the overall shape of the FD curve with radius. Two components of the shape curve were evident: a linear shape and a quadratic shape. This method avoided the problem of aligning radii the same in all patients. Figure 2B shows the mean overall fractal signature shape curves from patients with progressive OA and patients with nonprogressive OA. This method revealed decreased horizontal FDs (tension) and increased vertical FDs (compression) in patients with progressive OA compared with patients with nonprogressive OA, at particular regions of the curve.

Correlations with FSA. The remaining analyses were conducted using linear and quadratic fitted fractal signature data. Bivariate associations with fractal signatures are shown in Table 1. Linear shape (radius) and quadratic shape (radius²) were significantly associated with FDs. When linear and quadratic shapes were

combined with OA progression, there was a strong association with horizontal FD. Calcaneal BMD and BMC were also both associated with horizontal FD, and since the association was strongest in BMC, it was retained in lieu of BMD for subsequent analyses. Significant associations with vertical FDs were observed for linear shape and quadratic shape, as well as for sex, age, and BMI.

Prediction of OA progression based on global shape analysis of fractal signature curves. We next evaluated the prognostic ability of fractal signatures determined at baseline to predict OA progression status at 3 years, in models accounting for age, sex, BMI, BMC, knee pain, knee status at baseline, and knee alignment and adjusted using GEEs for the correlation between knees (Table 2). All fractal signature terms (horizontal and vertical, linear and quadratic) were determined in the medial subchondral region. Fractal signatures of the medial subchondral bone from baseline radiographs were significantly correlated with 3-year OA progression, based on JSN of the medial compartment. The baseline fractal signatures of the medial subchondral bone were not associated with OA progression based on osteophyte formation or with OA progression of the lateral knee compartment. In addition, age was independently predictive of medial and lateral JSN, while knee alignment was independently predictive of medial JSN. Accounting for these other factors, BMI was only independently predictive of lateral osteophyte progression.

Accuracy of fractal signatures for predicting OA progression. ROC curves were used to quantify the accuracy of predicting progression of medial JSN by

fractal signatures and by other variables individually and in combination. ROC curves were constructed to predict medial JSN using cross-validation in 5 groups. The null model is expected to have an area under the curve (AUC) of 0.5; 4 random variables resulted in AUCs of 0.50 (95% CI 0.41–0.57). The traditional covariates (age, sex, BMI) performed no better than the random variables for predicting OA progression, with AUCs of 0.52. The inclusion of BMC and knee pain as variables increased the power to predict OA progression only slightly (AUC 0.58 [95% CI 0.46–0.69]). Baseline OA status (JSN) alone was no more effective in predicting knee OA progression (AUC 0.52 [95% CI 0.44–0.59]) than the random variables. FSA had a remarkably good capability for predicting OA progression (AUC 0.75 [95% CI 0.65–0.84]), and the predictive ability of FSA did not improve with the inclusion of age, sex, BMI, BMC, and knee pain as covariates (AUC 0.74 [95% CI 0.65–0.84]). Among the other variables, only knee alignment was moderately predictive of medial JSN progression (AUC 0.68 [95% CI 0.57–0.81]). The best model with the fewest variables (AUC 0.79 [95% CI 0.72–0.88]) was not much better at predicting OA progression than FSA alone, and used age, sex, BMI, BMC, knee pain, knee alignment, and FSA, but not baseline OA status, as covariates. Six representative ROC curves are depicted in Figure 3.

Utility. To understand how FSA might benefit the design of future clinical trials, we extracted data from the ROC curves for this cohort to estimate the number of patients who would have to be screened in

Table 2. Prediction modeling of OA progression defined by JSN or osteophyte formation*

Factor	Outcome					
	JSN	Medial JSN	Lateral JSN	Osteophytes	Medial osteophytes	Lateral osteophytes
Left knee/right knee	0.397 (–0.307)	0.635 (–0.251)	0.317 (–0.530)	0.817 (–0.053)	0.673 (0.113)	0.908 (–0.028)
Age	0.384 (–0.020)	0.025 (–0.088)	0.032 (0.067)	0.419 (0.012)	0.978 (0.001)	0.719 (0.005)
Sex	0.654 (0.245)	0.589 (–0.473)	0.178 (1.019)	0.605 (–0.174)	0.554 (–0.239)	0.934 (–0.030)
BMI	0.352 (–0.042)	0.067 (–0.115)	0.503 (0.063)	0.068 (0.043)	0.241 (0.027)	0.036 (0.053)
Calcaneal BMC	0.387 (–0.062)	0.245 (0.153)	0.193 (–0.143)	0.896 (0.007)	0.988 (0.001)	0.681 (–0.024)
Knee pain	0.629 (–0.133)	0.110 (–0.669)	0.874 (–0.055)	0.108 (–0.301)	0.744 (–0.064)	0.286 (–0.203)
Knee alignment	0.119 (–0.113)	0.016 (–0.252)	0.610 (–0.057)	0.096 (0.045)	0.523 (0.018)	0.063 (0.060)
JSN status at baseline	0.120 (–0.377)	0.494 (–0.201)	0.167 (–0.533)	0.026 (0.372)	0.005 (0.592)	0.322 (0.171)
Vertical (compression) shape terms						
Linear	0.019 (–9.081)	0.010 (18.225)	0.631 (–1.701)	0.515 (–1.275)	0.129 (–3.235)	0.586 (–1.140)
Quadratic	0.025 (–7.531)	0.015 (58.807)	0.760 (–3.057)	0.508 (–3.941)	0.180 (–8.942)	0.535 (–3.896)
Horizontal (tension) shape terms						
Linear	0.042 (4.585)	0.012 (10.163)	0.721 (1.105)	0.893 (0.210)	0.191 (2.380)	0.626 (–0.752)
Quadratic	0.062 (10.926)	0.021 (24.153)	0.551 (4.495)	0.728 (–1.569)	0.474 (3.895)	0.371 (–4.133)

* Values are the *P* value (parameter estimate) determined with type 3 generalized estimating equation models. OA progression was calculated as fractal signature information (in polynomial parameters) + clinical covariates + design parameters + multiple correlation structures. See Table 1 for definitions.

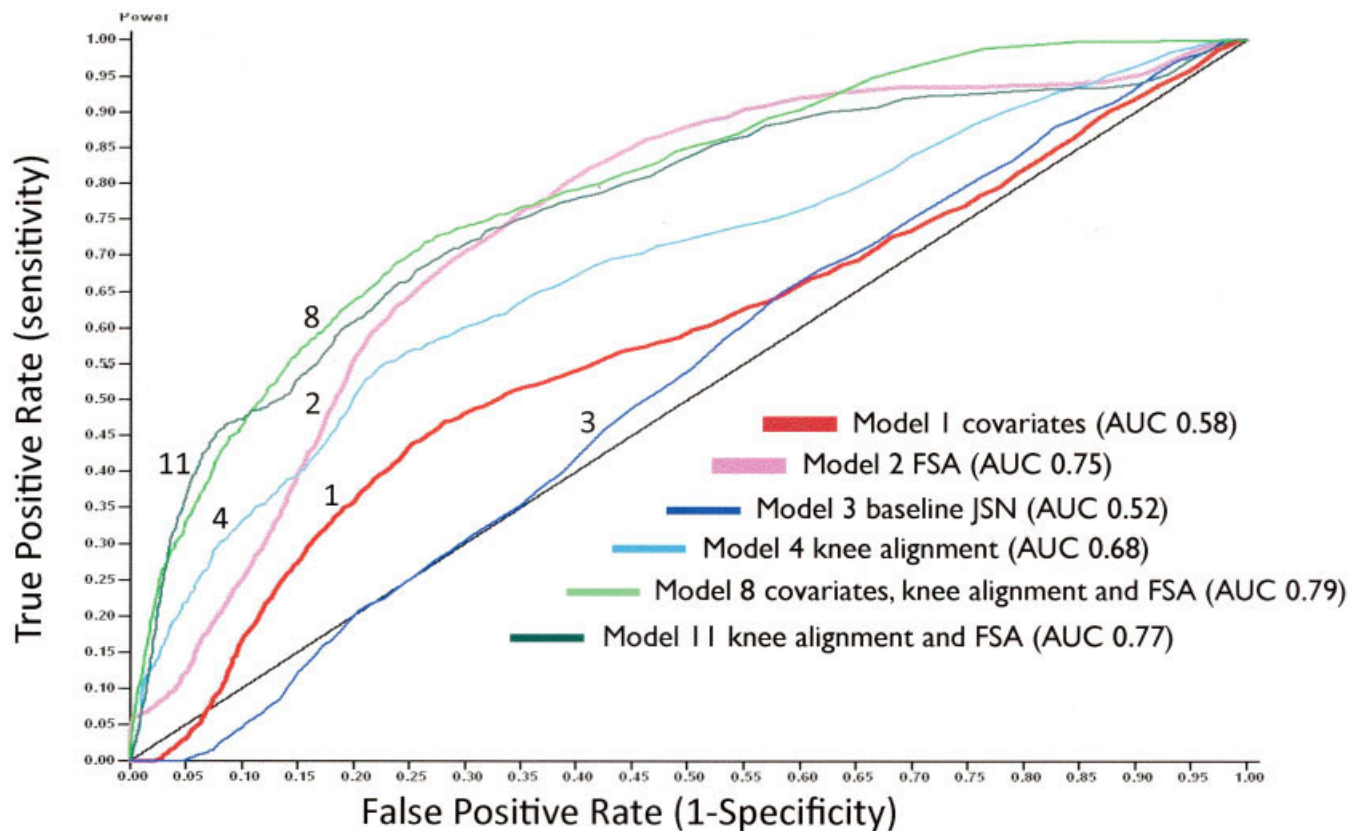


Figure 3. Representative receiver operating characteristic curves depicting the strength of the predictive models of medial osteoarthritis (OA) joint space narrowing (JSN). The black diagonal line represents the result of using random variables to predict progression of JSN in medial knee OA. In model 1, the covariates age, sex, body mass index (BMI), knee pain, and bone mineral content (BMC) were used as variables. In model 2, fractal signature analysis (FSA) alone was used. In model 3, medial JSN at baseline alone was used. In model 4, knee alignment alone was used. In model 8, the model with highest overall area under the curve (AUC), the covariates (age, sex, BMI, knee pain, and BMC) in combination with knee alignment and FSA were used. In model 11, knee alignment and FSA were used.

Table 3. Number of patients that must be screened in order to predict 1 patient with progressive medial JSN, using traditional covariates and FSA*

False-positive rate	No. of patients needed using covariates	No. of patients needed using FSA
0.01	∞	15.00
0.02	398.8	12.50
0.03	74.75	10.91
0.04	41.24	9.23
0.05	23.92	7.55
0.06	17.33	5.85
0.07	11.61	5.26
0.08	8.99	4.71
0.09	6.72	4.17
0.10	5.72	3.85
0.30	2.08	1.42
0.50	1.68	1.13
0.70	1.36	1.07
0.90	1.08	1.05

* JSN = joint space narrowing; FSA = fractal signature analysis.

order to identify 1 patient with progressive disease of the medial compartment. We compared the predictive ability of the traditional covariates (age, sex, BMI, knee pain) and BMC with that of FSA of the medial compartment. As demonstrated for a variety of false-positive rates, fewer individuals need to be screened in order to predict progressive OA using FSA than need to be screened using the other covariates. At a Type I error rate of 5%, 8 individuals would need to be screened using FSA, versus 24 using the other covariates, to identify 1 patient with progressive knee OA (Table 3).

DISCUSSION

Although trabecular structure is not truly fractal in nature, trabeculae possess fractal-like properties at a resolution similar to that of a plain radiograph (10). For

this reason, FSA is a valuable analytic tool for characterizing the complicated histomorphometry of bone. However, one of the major challenges in studies using FSA is how to analyze the complex fractal signature data. The most recent studies involving FSA and OA generally relied on a method whereby the mean fractal signature of OA patients or a treatment group is simply subtracted from that of a non-OA control or reference group (21,23,24). We found it was necessary to develop a method of analysis by which we could compare baseline data cross-sectionally to distinguish patients with progressive OA from patients with nonprogressive OA.

Our strategy was to focus on a global approach, curve fitting with a second-order polynomial regression. By using this approach, we found that OA progression defined by JSN was significantly associated with the shape of the fractal signature curves. Briefly, we found that higher fractal signatures of vertical trabeculae at baseline and lower fractal signatures of horizontal trabeculae at baseline could help us distinguish patients with progressive OA from patients with nonprogressive OA. FSA of bone texture of the inner three-fourths of the medial tibial compartment exhibited a 75% predictive capacity by ROC curve for predicting individuals with significant medial JSN but not osteophyte formation. These results could be useful to the design of future clinical trials, and we hope our findings will assist in other trials meant to identify patients with progressive JSN.

Independent of disease state, age has been shown to be associated with an increased number of fine vertical and horizontal trabeculae observed using FSA (14); in these past studies, the size of trabeculae affected by age did not overlap with the range of trabecular sizes altered by OA (16). Using the global shape analysis approach, we found that age had only a small effect on vertical FSA. Previously, no correlation between BMI and FSA had been found (17). Using our approach, we found that BMI had a small but significant effect on vertical FSA.

Although no previous studies have evaluated subchondral trabecular texture as a predictor of OA progression, 2 longitudinal studies using a subtractive approach (fractal signatures at followup minus fractal signatures at baseline) have shown that there is a significant change in trabecular texture coincident with OA progression (19,25). Both the direction of these fractal signature changes coincident with progression and the data from the subarticular region in particular are consistent with the differences we observed between patients with progressive OA and patients with nonpro-

gressive OA, differentiated at baseline. Another study showed a significant decrease in the horizontal FD within 4 years of a rupture of the ACL (27), suggesting that the bone changes following ACL rupture occur early and reflect those observed in progressive knee OA.

Previous studies using other approaches have shown that changes in periarticular bone occur very early in the development of OA, supporting the concept that skeletal adaptations precede detectable alterations in the structural integrity of the articular cartilage (42). It has been previously demonstrated that FSA detects changes in periarticular bone that are not discernible using DXA (9). Our data support the contention that changes in periarticular bone are high-sensitivity indicators of the disease process in human OA and provide a prognostic factor with high predictive capability for subsequent cartilage loss. One reason for this is the marked differential capacity of cartilage and bone to adapt to mechanical loads and damage (42). Cortical and trabecular bone rapidly alter skeletal architecture and shape in response to load via cell-mediated modeling and remodeling. Chondrocytes also modulate their functional state in response to loading. However, the capacity of these cells to repair and modify their surrounding extracellular matrix is relatively limited in comparison with skeletal tissues. This differential adaptive capacity likely underlies the more rapid appearance of detectable skeletal changes in OA, especially after injuries that acutely alter joint mechanics.

An increase in FDs, as observed in the current study in the vertical (compression) component in patients with progressive knee OA, has been equated with increased complexity of the image due to increased trabecular numbers secondary to thinning and fenestration of coarser trabeculae (i.e., a bone resorptive process). Decreased FDs, as observed in the current study in the horizontal (tension) component in patients with progressive knee OA, has been equated with decreased complexity of the image due to apparent decreased trabecular numbers secondary to trabecular coarsening manifested as horizontal striations on the radiograph (24). It has been speculated that changes in the horizontal component result from a thickened cortical plate and from retention of the horizontal trabeculae associated with enhanced absorption of load-bearing stress, resulting in reduced load transmission to the underlying trabecular bone, termed "stress shielding," and in the development of progressive osteoporotic change (22).

Curiously, in our study, the shapes of the vertical and horizontal FSA curves for the medial subchondral region of the knee appeared to differ from the shapes of

the curves in the study by Buckland-Wright and colleagues (17). In that study, the authors observed an initial increase followed by a steady decrease in the FD in both vertical and horizontal directions with increasing radius (17), while we observed a decrease in FD in the vertical trabeculae and an increase in FD in the horizontal trabeculae with increasing radius. In part, this difference may be explained by the fact that we did not use digitized macroradiographs, as was done in the previous work, so the smallest trabeculae were undetected in our analyses. The radial dimensions in the study by Buckland-Wright (0.06–1.14 mm) were also different from those in our analyses (0.4–3 mm). As such, our curves represent an extension encompassing larger radii compared with those published in previous reports and are in fact consistent with those found in the study by Buckland-Wright and colleagues (17,27). Additionally, our ROIs differed. In the study by Buckland-Wright in which the FSA curves are reported (17), the authors used an ROI spanning the outer three-fourths of the medial tibial plateau rather than the inner three-fourths of the plateau, as was used in the study by Messent et al (9) and in the current study. Nevertheless, the characteristic differences between fractal signature patterns of patients with progressive disease and patients with nonprogressive disease were comparable with the differences observed between patients with rapidly progressing disease and those with slowly progressing disease in the longitudinal study by Buckland-Wright and colleagues (19).

OA progression can be influenced by both systemic and local factors (43). Among the most potent risk factors for structural disease progression are limb malalignment (both static and dynamic) and obesity, the effect of which is mediated by malalignment (44). Although knee alignment did increase the effectiveness of FSA in predicting OA progression more so than traditional covariates, including BMI, the increase was only modest.

A number of physical characteristics observed using magnetic resonance imaging (MRI) have been associated with knee OA progression, including meniscal factors (1,45) and bone marrow lesions (46,47), as well as joint effusion, synovial pathology, cartilage lesions, and osteophytes (45). Observations of soft tissue made using MRI and observations of bone texture made using FSA of radiography have not been compared, so the relative strength of these predictors remains unknown. However, using MRI, Hunter et al found that the strongest predictor of disease progression in their study, meniscal subluxation, contributed 7% predictive

power to a model consisting of age, sex, and BMI (1), whereas we found that FSA alone contributed 75% predictive power and an additional 17% to a model consisting of age, BMI, sex, knee pain, and BMC. Furthermore, our results with FSA were derived by cross-validation, which is a reliable and conservative approach to estimating predictive power. We are not aware of any other individual predictive factor as good as FSA currently described in the literature. Moreover, both FSA and knee alignment can be obtained using a standard radiograph and could provide the most cost-effective means of maximizing the identification of patients with progressive disease.

FSA may be a valuable adjunct in OA clinical trials for several reasons. First, FSA can provide a quantitative indication of the effects of a drug on bone remodeling, especially drugs that have a direct effect on bone, including the bisphosphonates (25), calcitonin (48), diacerhein (49), and cathepsins (50). Second, the ability of FSA to reveal cartilage loss (19) and to predict risk for cartilage loss, as demonstrated in the current report, makes it a potential outcome measure for trials of drugs whose mechanism of action may be targeted to combat cartilage breakdown, not just bone remodeling. Third, FSA is relatively robust against differences in the acquisition and quality of radiographs and in the positioning of patients during radiography and could be readily completed using a standard knee radiograph, thus providing a cost-effective outcome measure that could be effectively instituted in a multicenter trial.

A limitation of our study was that we did not use macroradiographs. However, in a past study, radiographic method was shown to have no significant effect on the reproducibility of vertical and horizontal FSA; the only difference observed for macroradiographs was that they revealed differences between OA and control groups across a wider range of trabecular widths than those revealed using standard radiographs (23). As was done in past studies, we performed FSA on digitized images. We compared reliability and results of FSA from digitized and digital image formats and noted a loss of data from a few of the smallest radii only (data not shown), an effect that is likely due to the subtle smoothing of detail in the secondary digitization. It will be useful to determine in a future study whether the use of images acquired digitally may increase sensitivity for changes in very small radii and may further enhance the predictive capability of the FSA.

A strength of the current study was that we analyzed both left and right knees and controlled appropriately for correlation between knees, thereby increas-

ing study power. We also controlled for status at baseline and derived conservative estimates in the predictive models due to our cross-validation approach. Inclusion of all types of knee OA from our cohort revealed that medial tibial plateau fractal signature specifically predicted medial JSN. This compartmental specificity of FSA for ipsilateral JSN provides additional evidence for the face validity of FSA. It remains to be seen whether lateral FSA will have similar predictive capability for lateral JSN. The predictive capability of lateral FSA for lateral OA progression has not been assessed previously; all previous studies had patient populations with isolated medial compartment or medial compartment–dominant disease. Finally, our findings also suggest that traditional covariates and knee status at baseline are poor means of enriching a clinical trial of patients with progressive JSN.

In conclusion, Buckland-Wright recently suggested (51) that the 3 reasons to obtain a radiograph for research or clinical trial purposes are as follows: to establish the diagnosis or the degree of severity of OA, to monitor disease activity, progression, and possible therapeutic responses, and to look for complications of the disorder or therapy. With the promising FSA data that we report herein, we suggest that a fourth reason to obtain a radiograph might be to evaluate the risk of progression of knee OA. Using FSA to predict disease progression could augment an OA treatment trial in patients with progressive knee OA to help minimize trial cost and drug exposure and to increase the power of the study to show an effect.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Kraus had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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