Path Analysis Identifies Receptor Activator of Nuclear Factor-κB Ligand, Osteoprotegerin, and Sclerostin as Potential Mediators of the Tophus-bone Erosion Relationship in Gout

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ABSTRACT. ***Objective.*** To determine the relationship between tophus, erosion and bone remodeling factors in gout.

Methods. Computed tomography bone erosion and circulating bone factors were measured in adults with tophaceous gout. Multiple regression modeling and path analysis were used to determine predictors of erosion.

Results. Tophus number, Māori or Pacific ethnicity, creatinine, receptor activator of nuclear factor-κB ligand (RANKL), osteoprotegerin (OPG), and sclerostin were independently associated with erosion. Path analysis showed a direct effect of tophus number on erosion, partially mediated through OPG, RANKL, and sclerostin.

Conclusion. Tophus number is strongly associated with bone erosion in gout. Circulating RANKL, OPG, and sclerostin are potential mediators of tophus-related erosion. (First Release January 15 2016; J Rheumatol 2016;43:445–9; doi:10.3899/jrheum.150738)

Key Indexing Terms:
GOUT BONE EROSION RANKL OPG SCLEROSTIN

Tophi have been strongly implicated in the pathogenesis of bone erosion in people with gout[1]. Disordered bone remodeling also contributes to development of erosion; both enhanced osteoclastogenesis and impaired osteoblast viability and function have been described at sites of bone erosion in gout[2,3]. Osteoblast and osteoclast function are regulated by a number of soluble factors[4,5].

Path analysis is a special case of structural equation modeling. This method estimates the size of hypothesized causal connections between variables and enables comparison between different hypothesized sets of causal connections and provides a framework for choosing which of the putative pathways best fits the data through model goodness-of-fit index (GFI) statistics[6]. A key feature of path analysis is the ability to summarize a successful model with a path diagram that demonstrates a linear causal pathway among variables by unidirectional arrows extending from each of the determining variables to the variable dependent upon it. Indirect effects are the association of 1 variable with another mediated through other variables in the model. Direct effects are the associations demonstrated to be free of indirect paths. Path coefficients (standardized regression coefficients) included alongside each path (arrow) indicate the strength and direction of the association. Using path analysis, we aimed to determine the relationship between tophus, bone erosion, and soluble regulators of bone remodeling in gout.

MATERIALS AND METHODS

One hundred adults with tophaceous gout were prospectively recruited into a 2-year randomized placebo-controlled trial of zoledronate for treatment of erosive gout[7]. This trial was approved by the Northern X Regional Ethics Committee and all participants provided written informed consent. Among the inclusion criteria were diagnosis of gout according to the 1977 American

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Rheumatism Association classification criteria[8], at least 1 subcutaneous tophus, and stable urate-lowering therapy. Exclusion criteria included impaired renal function. At the baseline visit, subcutaneous tophus count, blood samples, and conventional computed tomography (CT) scans of both feet were obtained. Ankles and feet were scanned axially in 1 helical acquisition and bone erosion was scored by 2 musculoskeletal radiologists using a validated semiquantitative erosion score[9] (see Appendix 1).

The following soluble regulators of bone remodeling were measured in serum samples obtained at the same baseline visit by ELISA: free soluble receptor activator of nuclear factor-κB ligand (RANKL), osteoprotegerin (OPG, a soluble decoy receptor for RANKL), sclerostin (an osteocyte-derived Wnt inhibitor of bone formation), Dickkopf 1 (DKK-1, a Wnt inhibitor), and fibroblast growth factor 23 (FGF-23, an osteocyte-derived regulator of phosphorous and vitamin D). Bone turnover markers procollagen type-1 N-terminal propeptide (P1NP) and collagen cross-linked C-telopeptide, type I (CTX) were measured using the Roche Elecsys 2010 platform (see Appendix 1).

Bivariate associations between total number of tophi, regulators (RANKL, OPG, sclerostin, DKK-1, and FGF-23) and markers of bone remodeling (P1NP and CTX), age, disease duration, and serum creatinine and urate were tested by correlation (Pearson) with erosion score; and differences in erosion score were sought by sex and ethnicity (Māori or Pacific vs others, Student's t test; Table 1). A multiple linear regression model of variables chosen by external clinical review that could potentially be associated with bone erosion score was performed (with male sex and ethnicity dummy coded). A reduced model of variables that met the standard $p < 0.15$ criterion for inclusion was constructed to determine the subset of independent predictors of erosion score. Standardized β coefficients and variance explained (partial R^2) are presented for each variable (Table 2; see Appendix 1).

The PROC CALIS procedure (SAS v9.4, SAS Institute Inc.) was used to construct a mediation pathway to erosion score acting from the number of tophi to erosion score mediated through soluble bone remodeling regulators identified as potentially independent predictors of erosion score in the multiple regression analysis and adjusted for potential confounders. The direction of the path analysis from number of tophi to erosion score was prespecified. Total, direct, and indirect standardized effect estimates of the causal association were calculated and Sobel's test was used to test whether each of the putative mediator variables significantly carried the influence of an independent variable to a dependent variable; i.e., whether the indirect effect of the independent variable on the dependent variable through the mediator variable was significant.

RESULTS

Clinical features and correlation analysis are shown in Table 1. The mean (SD) erosion score was 17.1 (12.4). A strong correlation was observed between the number of tophi and erosion scores ($r = 0.62$, $p < 0.001$). Erosion scores positively correlated with circulating RANKL ($r = 0.44$, $p = 0.001$) and OPG ($r = 0.27$, $p = 0.006$), and negatively correlated with sclerostin ($r = –0.029$, $p = 0.003$). Erosion scores were higher in Māori and Pacific patients ($p = 0.03$), and a positive correlation was also observed between bone erosion and serum creatinine ($r = 0.34$, $p = 0.001$). No significant relationship was observed between bone erosion scores and the following variables: DKK-1, FGF-23, P1NP or CTX levels, age, sex, disease duration, or serum urate level ($p > 0.05$ for all).

Variables selected by external clinical judgment were included in a multivariable model and those variables with $p < 0.15$ were included in a reduced multivariable regression model (Table 2). In the reduced model, these were independently associated with erosion scores: number of tophi, Māori or Pacific ethnicity, serum creatinine, RANKL, OPG, and sclerostin ($F = 17.8$, $R^2 = 0.58$, $p < 0.0001$, Table 2). Because normality is an important underlying assumption of structural equation modeling, the distributions of the putative bone remodeling factors were tested for normality and RANKL

Table 1. Clinical characteristics and their correlations with CT erosion score. Data are mean (SD) unless indicated otherwise.

Clinical Features		Pearson r	P
Age, yrs	56 (12)	–0.05	0.66
Male sex, n (%)	94 (94)	—	0.24†
Ethnicity, n (%)		—	0.03†
Pacific	42 (42)		
Māori	18 (18)		
Non-Māori, non-Pacific	40 (40)		
Gout disease duration, yrs	22 (11)	0.20	0.051
No. subcutaneous tophi	7.3 (7.1)	0.62	< 0.001
Serum urate, mmol/l	0.38 (0.12)	0.22	0.29
Serum creatinine, μmol/l	97 (23)	0.34	0.001
RANKL concentration, pmol/l	0.28 (0.65)	0.44	0.001
OPG concentration, pg/ml	2214 (1272)	0.27	0.006
Sclerostin concentration, pg/ml	696 (338)	–0.29	0.003
DKK-1 concentration, pg/ml	3412 (1992)	–0.08	0.46
FGF-23 concentration, pg/ml	7.1 (11.8)	0.18	0.08
P1NP concentration, μg/l	56.9 (33.3)	0.16	0.12
β-CTX concentration, ng/ml	0.29 (0.16)	0.07	0.46

Pearson r and p refer to correlation with CT erosion score. †P value shown for comparison of erosion scores between groups. CT: computed tomography; RANKL: receptor activator of nuclear factor-κB ligand; OPG: osteoprotegerin; DKK: Dickkopf 1; FGF-23: fibroblast growth factor 23; P1NP: procollagen type-1 N-terminal propeptide; CTX: collagen cross-linked C-telopeptide, type I.

Table 2. Multiple linear regression analysis. Standardized β coefficient, partial R^2 and regression p are shown for those variables that met an inclusion criterion of $p < 0.15$ from a fully saturated model of variables selected from external clinical judgment, for an association with erosion score.

Clinical Features	Model Constructed from External Clinical Judgment		Reduced Model ($F = 17.8$, $R^2 = 0.58$, $p < 0.0001$)		
	Standardized β Coefficient	p	Standardized β Coefficient	p	Partial R^2
Age	–0.045	0.635			
Sex	0.023	0.769			
Māori or Pacific ethnicity	0.169	0.048	0.171	0.016	5%
Gout disease duration	0.088	0.259			
Total no. tophi	0.459	< 0.0001	0.460	< 0.0001	39%
Serum urate	0.057	0.469			
Serum creatinine	0.182	0.038	0.222	0.004	5%
RANKL concentration	0.184	0.041	0.209	0.009	3%
OPG concentration	0.142	0.119	0.158	0.036	2%
Sclerostin concentration	–0.168	0.028	–0.172	0.019	3%
DKK-1 concentration	–0.060	0.480			
FGF-23 concentration	0.012	0.892			
P1NP concentration	0.122	0.197			
β-CTX concentration	–0.222	0.033	–0.12588	0.103	

RANKL: receptor activator of nuclear factor-κB ligand; OPG: osteoprotegerin; DKK: Dickkopf 1; FGF-23: fibroblast growth factor 23; P1NP: procollagen type-1 N-terminal propeptide; CTX: collagen cross-linked C-telopeptide, type I.

subsequently replaced by rank-transformed RANKL for the path analysis.

A path analysis was performed to investigate putative causal pathways by estimating direct (tophus to erosion score) and indirect effects (tophus to erosion score mediated through bone regulators), controlling for Māori or Pacific ethnicity and serum creatinine. The model fit was excellent [$R^2 = 0.57$, $p < 0.001$, overall model fit chi-square = 130.2, $p < 0.0001$, goodness-of-fit index (GFI) = 0.93, parsimony adjusted GFI = 0.82]. Standardized path coefficients are shown (Figure 1). The direct effect of tophus number on erosion score was reconfirmed (independently 39% of variance). There were significant positive associations between the number of tophi, OPG, RANKL, and erosion score, and a significant negative association between sclerostin and erosion score (collectively 8% of explained variance). Sobel's test confirmed that the influence of tophus number on erosion score was significantly mediated through OPG ($p = 0.035$), RANKL ($p = 0.0021$), and sclerostin ($p = 0.044$; Figure 1).

DISCUSSION

In this study, tophus number had the greatest influence on CT erosion score (independently 39% of variance). Circulating

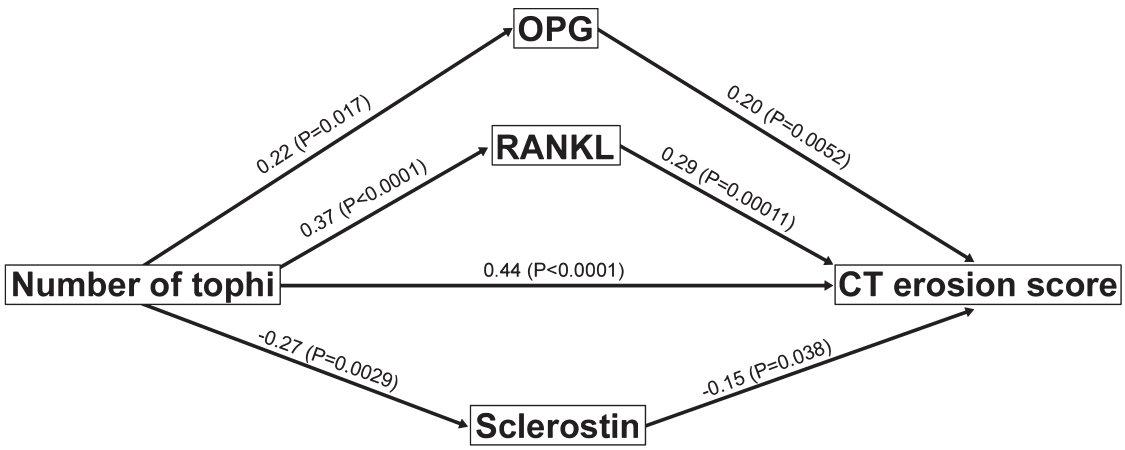

Figure 1. Standardized path coefficients (controlling for serum creatinine and ethnicity) from the path analysis investigating putative causal pathways of bone erosion in gout. Total $R^2 = 0.58$, $p < 0.0001$. RANKL: receptor activator of nuclear factor-κB ligand; OPG: osteoprotegerin; CT: computed tomography.

RANKL, OPG, and sclerostin levels were also associated with periarticular bone loss, suggesting a role in mediating tophus-related bone erosion. The data analysis indicated a direct influence (cumulatively 8% of explained variance) of bone remodeling regulators on CT erosion score independent of the number of tophi. The direction of the associations suggest that increasing tophus numbers act through RANKL to promote osteoclastogenesis in erosive gout, and that other soluble factors such as OPG and sclerostin may promote compensatory or repair mechanisms to maintain bone homeostasis at sites of monosodium urate (MSU) crystal deposition. New bone formation is also strongly associated with bone erosion and intraosseous tophus in gout[10], and it is possible that increased OPG and reduced sclerostin levels play a role in the development of these anabolic changes.

In addition to circulating concentrations of RANKL, OPG, and sclerostin, we observed that Māori or Pacific ethnicity and serum creatinine were independently associated with bone erosion scores in the reduced multivariate regression model. Māori and Pacific people have high prevalence of gout with early onset of disease and severe disease manifestations[11]. Impaired renal function is also associated with early onset of clinically apparent gouty tophi[12]. Importantly, the path analysis controlled for both ethnicity and serum creatinine.

A positive correlation between circulating RANKL and bone erosion has been previously reported in a separate group of patients with tophaceous gout[3]. These findings are similar to those of rheumatoid arthritis, in which patients with erosive disease have higher circulating levels of RANKL[13] and the RANKL:OPG ratio predicts radiographic progression[14,15]. The source of elevated circulating RANKL in patients with erosive gout is currently uncertain, but RANKL-expressing T cells within the tophus have been described[16], indicating that T cell–derived RANKL may promote osteoclastogenesis and bone erosion at sites of MSU crystal deposition. The osteocyte is a major source of RANKL, OPG, and sclerostin in the skeleton[17,18,19], and our data provide justification for research examining the role of the osteocyte in gouty erosion.

At present, the optimal strategy for prevention or treatment of bone erosion in tophaceous gout is uncertain. In the randomized controlled trial of zoledronate, despite improvements in bone mineral density and suppression of bone turnover markers, antiosteoclast therapy did not influence bone erosion in people with tophaceous gout on stable urate-lowering therapy[7]. In contrast, a small case series has reported that profound urate-lowering using pegloticase can improve erosion scores in patients with tophaceous gout and may promote healing of joints[20]. Our data also raise the possibility that, in combination with intensive urate-lowering therapy to prevent progression of bone erosion in tophaceous disease, targeting additional mediators of bone remodeling such as RANKL may also have therapeutic benefits.

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APPENDIX 1. Supplementary methods.

Patients. One hundred adults with tophaceous gout were prospectively recruited from rheumatology outpatient clinics into a two-year randomized placebo-controlled trial of zoledronate for treatment of erosive gout (7). This trial was approved by the Northern X Regional Ethics Committee and all participants provided written informed consent. Inclusion criteria included diagnosis of gout according to the 1977 American Rheumatism Association classification criteria (8), at least one subcutaneous tophus confirmed by a rheumatologist, and stable urate-lowering therapy. Exclusion criteria included impaired renal function (creatinine clearance <30 mL/minute).

Scoring of bone erosion using computed tomography. Bone erosion in the CT scans was scored by two musculoskeletal radiologists using a validated semi-quantitative erosion score (9). The gout CT bone erosion scoring system includes the following bones for erosion on a semi-quantitative scale from 0-10 in each foot: 1st metatarsal (MT) head, 2nd-4th MT base, cuboid, middle cuneiform, distal tibia (maximum total score 140). The radiologists were blinded to all clinical details, including clinical examination and laboratory results.

Measurement of soluble regulators of bone remodeling in serum. The following soluble regulators of bone remodeling were measured in serum samples obtained at the same baseline visit by ELISA: free soluble receptor activator of nuclear factor-κB ligand (RANKL; BioMedica Gruppe, Vienna, Austria), osteoprotegerin (OPG, a soluble decoy receptor for RANKL), sclerostin (an osteocyte-derived Wnt inhibitor of bone formation), Dickkopf-1 (DKK-1, a Wnt inhibitor) (all from R&D Systems, Minneapolis, MN), and fibroblast growth factor-23 (FGF-23, an osteocyte-derived regulator of phosphorous and vitamin D) (Merck Millipore, Billerica, MA). The CV was <5% for all assessed factors and the limits of detectability were as follows: 0.01pmol/L for RANKL, 156.25pg/ml for OPG, 125pg/ml for sclerostin, 125pg/ml for DKK-1 and 3.5pg/ml for FGF-23. The serum bone turnover markers pro-collagen type-1 N-terminal propeptide (P1NP) and β-C-terminal telopeptide of type I collagen (β-CTX) were measured using the Roche Elecsys 2010 platform (Roche Diagnostics, Indianapolis, IN).

Multiple regression modeling. Bivariate associations between total number of tophi, regulators (RANKL, OPG, sclerostin, DKK-1 and FGF-23) and markers of bone remodeling (P1NP and β-CTX), age, disease duration and serum creatinine and urate were tested by correlation (Pearson) with erosion score; and differences in erosion score were sought by gender and ethnicity (Māori or Pacific vs. others, Student's t-test) (Table 1). A multiple linear regression model of variables chosen by external clinical review that could potentially be associated with bone erosion score was performed (with male sex and ethnicity dummy coded). A reduced model of variables which met the standard P<0.15 criterion for inclusion was constructed to determine the subset of independent predictors of erosion score. Standardized beta coefficients and variance explained (partial R^2) are presented for each variable (Table 2). A sample size of >98 samples would have at least 80% power at the 5% significance level to medium sized effects of 0.2 and above.