Non-invasive Assessment of Failure Torque in Rat Bones with Simulated Lytic Lesions using Computed Tomography based Structural Rigidity Analysis

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INTRODUCTION: Strength of bone is determined by its material composition and structural organization. Pathologic fractures occur when the structural and/or material properties of bone are compromised to the degree that the bone can no longer withstand a given load. Based on the assumption that fragility fractures are caused by low bone mass, the World Health Organization (WHO) has identified individuals at risk for these fractures based on their areal bone mineral density (aBMD, g/cm²) measured by dual energy X-ray absorptiometry (DXA). However, DXA does not measure volumetric bone mineral density (vBMD, g/cm³) and does not distinguish changes in bone mineral composition from changes in bone structure and geometry. This distinction is important when diagnosing and treating skeletal pathologies associated with altered bone properties. Additionally, it has been shown that aBMD-based fracture predictions are neither sensitive nor specific for metastatic lesions.

In contrast, quantitative Computed Tomography (CT) based Structural Rigidity Analysis (CTRA), a 3D imaging modality, can provide information about specific changes in bone microstructure and tissue density for both cortical and trabecular bone. This technique can be used to non-invasively assess the axial, bending and torsional rigidities of bones from their trans-axial cross-sectional images. With this technique, modulus of elasticity is treated as a function of bone density, and bone geometry is represented by its cross-sectional area and moment of inertia. While CTRA has been used to assess fracture in studies of benign and metastatic musculoskeletal lesions in humans, the efficacy of this technique in assessing fracture risk in animal models of skeletal metastasis has not been characterized. Since osteolytic metastasis is associated with significant bone resorption and frequently leads to pathological fracture, we decided to use a simulated lytic defects model for this study.

Given the ability of CTRA to detect structural and material changes within bone, we hypothesize that CTRA-based failure torque correlates well with actual failure torque in bones with simulated lytic defects, and that CTRA is significantly more accurate than DXA-based aBMD measures in predicting failure torque. To that end, we aim to use CT-based Structural Rigidity Analysis to assess the failure torque of rat femurs with simulated lytic defects at different locations (proximal and distal femur) and diameters (25% and 50% of the cross-section of the site) and compare the results to those obtained from mechanical testing. Moreover, we aim to compare the correlation coefficients between CTRA-based failure torque and DXA-based aBMD versus actual failure torque.

MATERIALS AND METHODS: The study protocol was approved by Beth Israel Deaconess Medical Center’s Institutional Animal Care and Use Committee (IACUC). Twenty female Sprague Dawley rats (15 weeks old, mass= 250-275 g) were obtained from Charles River Laboratories (Charles River, Charlestown, MA, USA). One femur per animal, selected at random, was excised for the study. In order to simulate lytic defects, circular through holes were created in sagittal plane using a carbide drill bit under copious irrigation, while securing the bone in place using a padded vise. Four equally sized groups (n=5) were created based on different configurations of defect size and location: 1) Group A: Proximal defect of 25% femoral diameter, 2) Group B: Distal defect of 25% femoral diameter, 3) Group C: Proximal defect of 50% femoral diameter, 4) Group D: Distal defect of 50% femoral diameter.

Areal bone mineral density (aBMD, g.cm²) and bone mineral content (BMC, g) in the anterior-posterior direction were measured at the site of the defect using DXA (Lunar PIXImus2, General Electric, Waukesha, WI, USA). Sequential transaxial images through the entire non-embedded bone specimens were acquired using microcomputed tomography (µCT40, Scanco Medical AG, Brüttisellen, Switzerland) at an isotropic voxel size of 20 µm, integration time of 250 ms and tube voltage and current of 55 kVp and 145 µA respectively. Bone mineral density was calculated using a hydroxyapatite phantom to convert X-ray attenuation coefficient (µ) to an equivalent bone mineral density. Torsional rigidity (GJ) describes the structural behavior of a bone and its resistance to deformation when subjected to torsional loading. The failure torque was calculated based on the equation: GJ = π·E·I/4, where E is the Young’s modulus obtained from CTRA imaging and other specimen-specific. Following µCT imaging, a torsion system designed for non-homogeneous orthotropic or non-axisymmetric specimens that accommodates out of plane warping and bending was used for this study. Specimens underwent angular displacement controlled torsion to failure at a rate of 0.083 rad.s⁻¹. Angular displacement (θ), rad) and torque (T, N.m) were recorded for the duration of each test.

RESULTS: All specimens subjected to pure torsion fractured through the defect in a spiral line of fracture. CTRA-based failure torque was highly correlated with mechanical testing based failure torque results (T_{mech} = 0.91·T_{CTRA} + 0.044, R² = 0.85, P < 0.001). Additionally, analysis results demonstrated that the slope and y-intercept of the failure torque regression line were not different from the identity line (y = x) (P = 0.46), suggesting that the correlation between the CTRA-based failure torque and actual failure torque, as assessed by mechanical testing, were not skewed. The aBMD could only to describe 32% of the variation in the actual failure torque [aBMD = 2.22 · T_{mech} = 0.234, R² = 0.32, P = 0.054]. Multiple regression with defect size and location and actual failure torque as independent variables and CTRA-based failure torque as dependent variable indicated a strong correlation between the two failure torques (adjusted R² = 0.83, P < 0.001), where defect size (P = 0.43) and location (P = 0.95) had no effect on the correlation. Using Fisher’s Z-transformation test, the coefficients of determination for the linear regressions where actual failure torque was the dependent variable and CTRA-based failure torque was the independent variable were significantly better than the linear regression where DXA-based aBMD was the independent variable (P < 0.001). Failure torque differences between actual mechanical testing and CTRA methods were calculated for all pairs of data. There were no differences between the 4 groups (A, B, C, D) with respect to the average difference between actual mechanical and CTRA based failure torques (P = 0.82). Overall, the mean difference was essentially zero, but the standard deviation of the difference was 0.75 N. Thus, a Bland-Altman method would indicate that the 95% confidence interval around the difference is ±1.5 N as an estimate of the accuracy and error associated with predicting failure load.

DISCUSSION: Non-invasive prediction of failure torque in a simulated osteolytic rat model is aiming to answer a clinically relevant question, although these results should be interpreted with some considerations regarding this model. Naturally, bone metastasis starts by homing of tumor cells in the bone marrow sinusoids with initial involvement of trabecular bone and later extension to cortical areas. We have simulated this phenomenon by creating a symmetric through hole in the bone. Additionally, our model is limited to two anatomical locations across a single bone, where bones are tested under torsional loading. We acknowledge that lytic lesions occur in a variety of locations across the skeleton and are subjected to complex mechanical loading conditions. Despite these limitations, we believe that this model provides useful insight into the ability of CTRA to predict failure torque in bones with osteolytic lesions. The current gold standard for fracture risk assessment is DXA, which measures the average bone mineral content and density in a 2D projected area without the ability to differentiate changes in bone microstructure with those in bone tissue density. In contrast, computed tomography provides an accurate measurement of the changes in both bone tissue mineral density and microstructure in cancellous and cortical bone. The results of our study suggest a considerable potential for using CTRA in animal models to non-invasively assess failure torque in bones with lytic defects.