Raman Spectroscopy and Reference Point Indentation: Investigating the Predictive Value for Fracture Risk in Femoral Necks

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INTRODUCTION**:** Screening rates for osteoporosis (OP) remain low despite the alarming estimates that 33% of women and 20% of men will experience a fragility fracture in their lifetime [1]. Further, bone mineral density (BMD), the basis for DXA diagnosis of OP, only explains 21% of all non-vertebral fractures in men and 44% in women [1]. Therefore, there is an increasing need for more sensitive and reliable tools to assess bone fracture risk. Raman spectroscopy, capable of molecular fingerprinting, has been used in identifying the quality of the mineral (hydroxyapatite) and matrix (collagen) components of bone [2]. While fracture risk can be estimated based on DXA T-scores and fracture risk assessment tools such as FRAX® [3], it remains to be established whether Raman compositional parameters correlate with fracture risk. To investigate this, we used Raman spectroscopy and reference point indentation (RPI) to measure Raman spectra and the mechanical properties, respectively, of femoral necks from total hip arthroplasty patients. We then used the FRAX® tool [3] to assess the individuals' fracture risk. We hypothesized that Raman spectroscopy and RPI mechanical outcomes can predict a patient's fracture risk.

METHODS**:** Femoral necks were obtained from patients undergoing total hip arthroplasty at the University of Rochester Medical Center (n = 60) with IRB approval. The samples were wrapped in PBS-soaked gauze and stored at -80°C until further analysis. Each sample was divided into four quadrants: Inferior, superior, anterior, and posterior. To the best of our ability, we identified the quadrant with the thickest cortex as the inferior quadrant, which provided the most reliable measurements. We used DXA to measure the bone mineral density (BMD) and used Raman spectroscopy and reference point indentation (RPI) to measure Raman compositional parameters and the mechanical properties, respectively, in the same quadrants. The samples were thawed at room temperature before testing. To measure the Raman signal, the samples were excited using an 830nm, 150mW laser, and the scattered light was collected. Each spectrum was an average of 5 spectra with an integration time of 60 seconds. A custom MATLAB code was used to calculate the Raman outcomes (Table 1) [3]. For RPI (BioDent, Active Life Sciences), the samples were rehydrated in PBS for 2 hours before testing. Samples were tested in the same quadrants as Raman with an indentation force of 10 N and a frequency of 2.0 Hz for a total of 10 cycles. The quadrants with substantial loss of cortical bone were excluded from the analysis. Finally, the ten-year probability of a major osteoporotic and hip fractures were calculated using the FRAX® tool by inputting the patient characteristics from their medical charts. Principal component analysis was performed to reduce the dimensionality of the data. Statistics: A one-way analysis of variance adjusted with Tukey HSD was performed to test quadrant differences. Multivariate regression models with the principal components were then used to test the statistical associations between the predictor variables (Raman and RPI) and the FRAX® probabilities.

Table 1. Raman spectroscopy outcomes and the corresponding vibrational modes. [3]

Raman Outcomes	Vibrational modes
Mineral to matrix ratio (M:M)	ν_1PO_4/Amide III
Carbonate to phosphate ratio (C:P)	ν_1CO_3/ν_1PO_4
Mineral maturity/ crystallinity (MMC)	1/FWHM ν_1PO_4
Pyridinoline content (Pyd)	1660/Amide I

RESULTS**:** Femoral neck specimens were obtained from 32 male and 28 female THA patients with an average age of 66.4±9.2 years and an average BMI of 30.7±5.2 kg/m^2. Since measurements in the inferior quadrants were the most reliable, all the correlations described herein are based on measurements in that quadrant. The M:M trended towards being lower, whereas the C:P and Pyd increased with age, but not sex or BMI. None of the RPI outcomes correlated with age, sex or BMI. Multivariate regressions showed that Raman PC1 (-0.59 × M:M + 0.56 × C:P + 0.55 × Pyd) was a significant explanatory variable for several RPI outcomes. Interestingly, BMD did not correlate with FRAX® outcomes, but predictably, we estimated higher FRAX® probabilities in females, which also correlated with age but not BMI (Fig. 1). Further, multivariate regressions of the 4 Raman principal components revealed significant correlations with FRAX® ten-year probability of a major osteoporotic fracture (R^2=0.19). When stratified by sex, the regressions improved to R^2=0.245 for females and R^2=0.223 for males, respectively (Fig. 2). However, in females, PC2 (0.98 × crystallinity) had the highest correlative weight (p=0.06), whereas PC1 (-0.59 × M:M + 0.56 × C:P + 0.55 × Pyd) had the highest correlative weight in males (p<0.05). Similar correlations could be observed for the FRAX® ten-year probability of a hip fracture, although the model was not significant for females. Multivariate models based on RPI principal components failed to yield significant correlations with FRAX®.

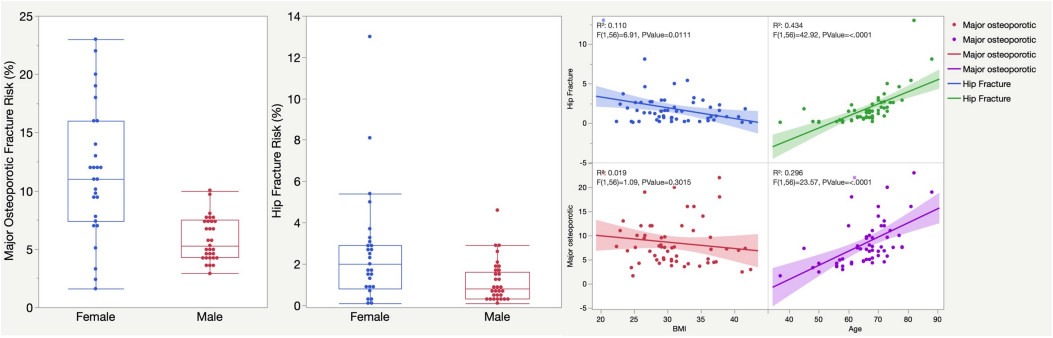

Figure 1. FRAX® ten-year probability of a major osteoporotic fracture or a hip fracture by sex, BMI, and age.

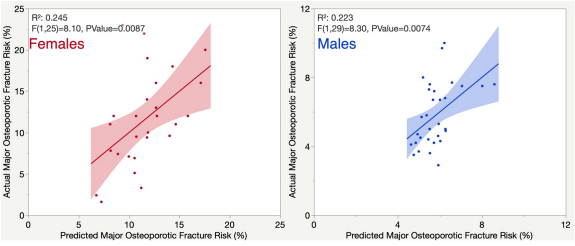

Figure 2. Multivariate correlations between Raman principal components and FRAX® ten-year probability of a major osteoporotic fracture, stratified by sex.

DISCUSSION**:** Our findings highlight the potential of Raman spectroscopy for assessing fracture risk in osteoporosis patients, underlining its ability to provide information beyond traditional BMD measurements obtained via DXA. Interestingly, BMD did not show a significant correlation with FRAX® outcomes, suggesting the need for additional metrics in fracture risk assessment. Raman principal components, based on classic compositional parameters, displayed significant correlations with FRAX® ten-year probabilities, particularly when stratified by sex, thereby supporting our initial hypothesis. This work shows promise in introducing new, more sensitive markers for osteoporosis risk stratification. The limited correlations between RPI mechanical outcomes and FRAX® suggest that further research is needed to understand the role of mechanical properties in bone fracture risk. Overall, this study offers preliminary evidence towards the incorporation of Raman spectroscopy as a potentially more reliable metric for osteoporosis screening and management.

SIGNIFICANCE/CLINICAL RELEVANCE**:** DXA BMD gives a measure of mineral content, whereas Raman compositional outcomes give an estimate of various compositional parameters. Given the low power of BMD in predicting fractures, Raman spectroscopy can be an effective alternative or adjunct tool.

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