ORIGINAL ARTICLE

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Site-Dependent Reference Point Microindentation Complements Clinical Measures for Improved Fracture Risk Assessment at the Human Femoral Neck

Thomas Jenkins,[1*] Louise V Coutts,[1*] Stefania D'Angelo,[2] Douglas G Dunlop,[3] Richard OC Oreffo,[4] Cyrus Cooper,[2,5,6] Nicholas C Harvey,[2,6**] Phillipp J Thurner,[1,7**] and the Observational Study Examining Osteoporosis (OStEO) group

[1]Bioengineering Science Research Group, Faculty of Engineering and the Environment, University of Southampton, Southampton, UK
[2]MRC Lifecourse Epidemiology Unit, University of Southampton, Southampton, UK
[3]University Hospital Southampton NHS Foundation Trust, Southampton, UK
[4]Bone and Joint Research Group, Centre for Human Development, Stem Cells and Regeneration, Institute for Development Sciences, Faculty of Medicine, University of Southampton, Southampton, UK
[5]NIHR Musculoskeletal Biomedical Research Unit, University of Oxford, Oxford, UK
[6]NIHR Biomedical Research Centre, University of Southampton and University Hospital Southampton NHS Foundation Trust, Southampton, UK
[7]Institute for Lightweight Design and Structural Biomechanics, TU Wien, Vienna, Austria

ABSTRACT

In contrast to traditional approaches to fracture risk assessment using clinical risk factors and bone mineral density (BMD), a new technique, reference point microindentation (RPI), permits direct assessment of bone quality; in vivo tibial RPI measurements appear to discriminate patients with a fragility fracture from controls. However, it is unclear how this relates to the site of the most clinically devastating fracture, the femoral neck, and whether RPI provides information complementary to that from existing assessments. Femoral neck samples were collected at surgery after low-trauma hip fracture ($n = 46$; 17 male; aged 83 [interquartile range 77–87] years) and compared, using RPI (Biodent Hfc), with 16 cadaveric control samples, free from bone disease (7 male; aged 65 [IQR 61–74] years). A subset of fracture patients returned for dual-energy X-ray absorptiometry (DXA) assessment (Hologic Discovery) and, for the controls, a micro-computed tomography setup (HMX, Nikon) was used to replicate DXA scans. The indentation depth was greater in femoral neck samples from osteoporotic fracture patients than controls ($p < 0.001$), which persisted with adjustment for age, sex, body mass index (BMI), and height ($p < 0.001$) but was site-dependent, being less pronounced in the inferomedial region. RPI demonstrated good discrimination between fracture and controls using receiver-operating characteristic (ROC) analyses (area under the curve [AUC] = 0.79 to 0.89), and a model combining RPI to clinical risk factors or BMD performed better than the individual components (AUC = 0.88 to 0.99). In conclusion, RPI at the femoral neck discriminated fracture cases from controls independent of BMD and traditional risk factors but dependent on location. The clinical RPI device may, therefore, supplement risk assessment and requires testing in prospective cohorts and comparison between the clinically accessible tibia and the femoral neck. © 2015 American Society for Bone and Mineral Research.

KEY WORDS: INDENTATION (MICRO); FRACTURE RISK ASSESSMENT; OSTEOPOROSIS; DXA; AGING

Introduction

The conceptual definition of osteoporosis incorporates loss of bone mass and deterioration of microarchitecture.[1] To facilitate epidemiological research, the World Health Organization (WHO) developed an operational definition based on bone mineral density, measured at the femoral neck or lumbar spine by dual-energy X-ray absorptiometry (DXA), which has subsequently become the

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Address correspondence to: Phillipp J Thurner, MSc, PhD, Institute for Lightweight Design and Structural Biomechanics, Vienna University of Technology, Getreidemarkt 9, 1060 Vienna, Austria. E-mail: pthurner@ilsb.tuwien.ac.at
*TJ and LVC are the joint first authors.
**NCH and PJT are the joint senior authors.
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NK Ardend,[5,6] JM Latham,[3] P Taylor,[3] M Baxter,[3] N Moss,[8] C Ball,[3] K Chan[9]
[2]MRC Lifecourse Epidemiology Unit, University of Southampton, Southampton, UK; [3]University Hospital Southampton NHS Foundation Trust, Southampton, UK; [5]NIHR Musculoskeletal Biomedical Research Unit, University of Oxford, Oxford, UK; [6]NIHR Biomedical Research Centre, University of Southampton and University Hospital Southampton NHS Foundation Trust, Southampton, UK; [8]Portsmouth Hospital NHS Trust, Portsmouth, UK; [9]University College London, London, UK.
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standard definition used in clinical care.[2] Over recent years, use of bone mineral density (BMD) has been combined with clinical risk factors in the calculation of absolute fracture risk, with FRAX being the most widely used algorithm globally. These approaches assess altered bone mass and non-BMD risk factors for fracture but provide only limited information on bone microarchitecture and mechanical integrity. Several techniques are now available to assess micro-architectural parameters, including high-resolution peripheral quantitative computed tomography (HRpQCT)[3] and trabecular bone score.[4] Although these techniques may provide some indirect assessment of overall bone strength, they do not allow direct measurement of bone material properties, clearly important in terms of susceptibility to fragility fracture.

Reference point indentation (RPI), also referred to as micro-indentation, is a novel technique that allows such direct measurement of bone material or biomechanical properties.[5,6] Currently, preclinical (Biodent, cyclic indentation to measure the indentation depth) and clinical devices (Osteoprobe, a single rapid indentation that measures the bone material strength [BMS] without using a physical reference probe, approximately inversely related to indentation depth) have been developed. Clinical RPI measurements are made at the tibia and can discriminate fracture cases from non-fracture controls.[5] Furthermore, RPI measured at the tibia appears to be independent of BMD[5,7,8] but does correlate with traditional biomechanical measures of bone strength in vitro,[5,8–12] suggesting that RPI may add usefully to information garnered from BMD and traditional risk factors.

Importantly, it is unclear how RPI measures might differ between fracture patients and controls at a key fragility fracture site, the femoral neck. We, therefore, hypothesized that RPI-measured indentation depth would be greater in femoral neck bone samples from hip fracture patients compared with samples from non-fractured cadaveric controls, and, additionally, we examined whether RPI may yield information adjunctive to that from BMD and FRAX probability.

Materials and Methods

Human bone samples

Human femoral neck samples were collected to form two groups: 1) the osteoporotic (OP) fractured group (46 samples) and 2) the cadaveric control group (16 samples). The osteoporotic group consisted of resected proximal femora (ie, femoral head and neck, Fig. 1*B*) from patients undergoing hip arthroplasty at University Hospital Southampton NHS Foundation Trust (UHS) for an intracapsular fracture of the femoral neck. Innoved Institute LLC (Besenville, IL, USA; 15 samples) and IIAM (International Institute for the Advancement of Medicine, Edison, NJ; 1 sample) provided frozen unembalmed samples to form the control group. Samples consisted of the proximal third (ie, Fig. 1*A*), cut from the whole femora that had been removed from cadaveric donors with no known history of fracture or bone disease.

Reference point microindentation

RPI was performed using the Biodent Hfc device (Active Life Scientific, Santa Barbara, CA, USA), which has been described in detail.[5,6,13] We have previously demonstrated that there is no optimal maximum load in terms of coefficient of variation but that higher loads may be more representative of the bulk properties of the bone.[13] Hence, we used 10 N (1300 g to 1350 g preload) over 10 cycles (for consistent indentation depth[9,14]) and cycling at 2 Hz. In the interest of concision, we present three parameters because of their prevalence in the literature (ie, indentation distance increase [IDI]) and efficacy in discriminating fractured from non-fractured tissue (total indentation distance [TID] and first cycle creep indentation distance [CID], also referred to as CID1) as described previously.[5,6,13] Many other indentation parameters have previously been presented in the literature (eg, Granke and colleagues[9] and Setters and Jasiuk[14]), eight of which were considered in this analysis. The loading (LS) and unloading (US) slope of the curves in particular were less effective at discriminating fracture cases from controls ($p > 0.05$ before adjustment). Last cycle or average CID and first cycle indentation distance (ID1) were similar to but also less effective than ($p > 0.1$ after adjustment) CID and TID as presented. Additionally, energy dissipated (ED) was reliant on indentation curve shape rather than clearly defined points and was, therefore, considered to be a less robust measurement.

Femoral neck testing procedure

The frozen (–80°C) proximal femoral samples were defrosted (overnight for more than 15 hours at room temperature), where

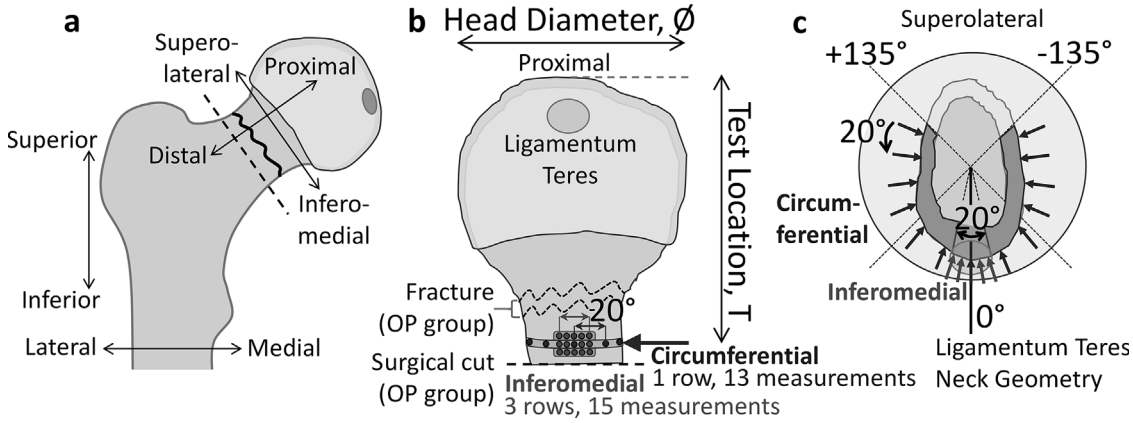

Fig. 1. Femoral neck indentation showing (*A*) the proximal femur, (*B*) the femoral head and proximal-distal testing location of the femoral neck, and (*C*) the circumferential (excluding the superolateral quadrant) and inferomedial testing locations of the femoral neck.

submerged in Hank's balanced salt solution (HBSS) for rehydration. Owing to the curved and irregular geometry of the femoral neck and the requirement to achieve an indentation angle within 10° of the normal,[15] the RPI device had to be held freehand (potentially with associated variability[13]). The site of femoral neck testing was standardized by measuring a set distance (test location, T, Fig. 1*B*), equal to the diameter of the femoral head (head diameter, Ø, Fig. 1*B*). However, this proximal-distal test location (ie, T/Ø = 1) was not always achievable because of the presence of the fracture in the osteoporotic group. The orientation of the samples was established through identification of the inferomedial point, where the cortex was thickest,[16] through the geometry of the femoral neck and through the location of the ligamentum teres (that stabilizes the femoral head to the acetabulum) as shown in Fig. 1*B, C*.

A scalpel was used to remove the majority of the soft tissue, and the bone surface was scratched with the probe as necessary to displace any remaining periosteum before testing, intending to minimize the effect of soft tissue (reduction of IDI, TID, and CID by 30% to 35% and a potential reduction in coefficient of variation[13]). The surface of the bone was not machined, with a view to mimicking the clinical test environment, albeit at the cost of potentially increasing coefficient of variation.[13]

Fig. 1*C* indicates that the probe was applied every 20° (ie, approximately 5 mm spacing, substantially greater than the minimum 500 μm[13]) circumferentially around ¾ of the femoral neck toward the inferomedial point giving 13-repeat measurements, thus reducing the error from the "true" value to less than 7%.[13] The location of the osteoporotic fracture limited the number of repeat measurements in some cases. The superolateral quadrant was excluded (Fig. 1*C*) where thickness (less than 10 times the indentation depth resulting from a 10 N load[13,16]) and porosity made indentation unreliable.[13,16–18] A subset (34 osteoporotic, 16 control) was indented in the inferomedial femoral neck where the cortex was thickest[16,18] and avoided porous regions.[17] Fifteen measurements were made within ±10° of the inferomedial point along three rows with 1 to 2 mm spacing (Fig. 1*B, C*).

Bone mineral densitometry

Nineteen fracture group participants underwent BMD DXA scans of the proximal femur at the Osteoporosis Centre, UHS, using a Hologic Discovery A instrument (Hologic Inc., Bedford, MA, USA). For the control group, radiographs were captured of the distal third of the proximal femora at the μ-VIS computed tomography center, University of Southampton, using a 225kV X-TEK/Nikon Metrology HMX ST system to reproduce the clinical DXA technique as described by Coutts and colleagues.[19] In brief, radiographs were taken at the same two energies (100 keV and 140 keV) and in the same anteroposterior view as the clinical scans, then the Beer-Lambert equations were applied to derive a bone mass map. In the same process as the clinical Hologic scanner, the areal FN BMD was calculated as an average density (bone mineral content divided by the area) over an area 10 mm proximal to the greater trochanter and perpendicular to the neck angle. A calibration was performed across the range of thicknesses (and hence range of known densities that incorporate that of bone) of a cylindrical aluminium rod, the calibration phantom. Further cross-calibration was then performed and the technique was found to be in good agreement with both the clinical Hologic scanner (aluminium calibration bar density within 2% of the known or HMX ST measured density) and the known weight (determined via weighing) of six proximal femoral samples ($r = 0.89$, $p = 0.017$ and mean densitometry/weighed mass = 0.97) with these details presented by Coutts and colleagues.[19]

Clinical risk factors

Clinical information including relevant medical history, medication, lifestyle factors (smoking history, exercise, and approximate alcohol, caffeine, and calcium consumption) and other factors relevant to bone health (height, weight, family history, fracture history, falls history) were collected where possible. For the fracture group, these data were collected through researcher-led questionnaires and access to the participants' medical notes. For the control group, these data were provided by the tissue bank through the "donor summary," based on the donor's medical notes. Using this patient information, FRAX 10-year hip fracture probability[20,21] was generated. The FRAX tool was used with clinical risk factors alone or in combination with femoral neck BMD where available.

Full IRB and ethics approvals were obtained for the study (LREC 194/99/1; 210/01; 12/SC/0325) from Southampton and South West Hampshire Research Ethics Committee.

Statistical analysis

Normality was not observed in repeat RPI measurements, so a median value was calculated to give a single measurement per donor. Using these single values, the distribution was also considered across donors and the osteoporotic fracture and control groups were compared. The mean and standard deviation are presented for normally distributed parameters (ie, IDI, CID) and *t* tests and Pearson's correlations used. The median and interquartile range are presented for non-normally distributed variables (ie, TID); Mann-Whitney *U* tests and Spearman's correlations were performed and these variables were then normalized using a Fisher Yates transformation for the linear regression models.

Three models were explored using multivariable linear regression: model 0 was unadjusted; model 1 included age, sex, BMI, and height; and model 2 additionally incorporated total sample storage time, proximal-distal test location, and the number of repeat measurements. Statistical significance was accepted as $p < 0.05$. Performance for discrimination of fracture cases from controls was undertaken using receiver-operating characteristic (ROC) analyses to calculate area under the curve (AUC) values. Supplementation of RPI to clinical factors (CRF) was performed by ROC analyses using two methods. Normalization (eg, the lowest TID value and highest BMD = 0 and the highest TID value and lowest BMD = 1) then summation gave a combined score (RPI + CRF: from 0, implying a low fracture risk, to 2, implying a high fracture risk) for ROC analysis. Alternatively, the clinical thresholds for BMD or FRAX were first applied followed by consecutive ROC analyses using RPI in those cases not identified by these thresholds (CRF then RPI). Statistical analyses was performed using SPSS v20 (IBM, Portsmouth, UK) and Stata v13.1 (StataCorp LP, College Station, TX, USA).

Results

Participant characteristics

Forty-six donors were recruited to the osteoporotic fracture group with a median age of 83 years (IQR = 77 to 87), and 16

samples were obtained to form the control cohort with a median age of 65 years (IQR = 61 to 74). The fracture group was older, had a lower BMI, a more distal test location, and a shorter storage period than the control ($p < 0.01$), as shown in Table 1, and all donors were of white race.

RPI measurement in fracture and controls

In model 0 (unadjusted), the circumferential indentation depth, measured in microns, was significantly greater in the osteoporotic fracture cases than control group (by 24.0% to 31.9% in terms of TID, IDI, and CID) as shown in Fig. 2 and Table 2 (Circ.).

In the control group, RPI had some correlation with age (TID, $r = -0.57$, $p = 0.022$, and CID $r = -0.51$, $p = 0.044$). In the fracture group, RPI was 18% greater in females than males (CID, $p = 0.023$) and a partial negative association with height (CID, $r = -0.38$, $p = 0.023$), number of measurements (TID, $r = -0.41$, $p = 0.004$) and test location (decreasing moving distally, $r = -0.31$ to -0.38, $p < 0.05$), and positively with BMI ($r = 0.35$, $p = 0.040$). Incorporating these factors into the multivariate analyses (Table 2), the greater indentation depth in the fracture than control group remained significant (TID, IDI, and CID, $p < 0.01$) when adjusted for patient-related parameters (model 1). When further adjusted for testing-related parameters (model 2), this difference just failed to achieve statistical significance for TID ($p = 0.051$) and CID ($p = 0.081$), however, this was non-significant for IDI ($p = 0.30$).

RPI and clinical measures

The correlation with RPI and BMD ($r = -0.39$ to 0.20) or FRAX ($r = -0.37$ to 0.29) alone varied across measures (TID, IDI, and CID) or cohorts (fracture and control), but in all cases was not statistically significant ($p > 0.05$). The FRAX hip fracture probability incorporating BMD was significant in the control group (CID, $r = -0.51$ and TID, $r = -0.62$, $p < 0.05$) but still did not completely categorize RPI, suggesting an independent contribution to fracture case discrimination. Fig. 3 demonstrates the ROC curves for RPI measures alone and in combination with FRAX and/or BMD. The ROC AUC values for RPI alone were high (0.79 to 0.89), which is also indicated by the degree of overlap of the distributions in Fig. 2*B*, but these were further improved through normalization and summation with clinical factors to create a combined score (ROC AUC = 0.88 to 0.99, RPI + CRF, Fig. 3 and Table 3). Furthermore, when considering those that were not currently identified by clinical factors (ie, 60% to 86%, that is, 100% minus the sensitivity shown in Table 2), RPI performed similarly well, offering discrimination in this currently unidentified group, albeit in a smaller number of samples (RPI with CRF, Fig. 3: ROC AUC = 0.78 to 0.94, Fig. 3 and Table 3). Supplemental Table S1 indicates that when considering many other risk factors (ie, hip structure analysis, HRpQCT measures, serum vitamin D and calcium levels, total spinal and TH DXA measures, and patient reported alcohol/caffeine/calcium consumption, quality of life, falls risk, and the FRAX incorporated factors), there were very few significant relationships ($p < 0.05$ for 6 of 189 relationships), and these measures only explained a small proportion of RPI ($r_{max} = 0.54$ and $|r| < 0.4$ in 125 of 141 cases).

Table 1. Baseline Characteristics for Osteoporotic Fractured (OP) and Control Groups

			OP	Control	*p* Value
Model 2	Model 1	Age (years)*	83 (77–87)	65 (61–74)	0.001
		Sex (F:M)	29:17 (1.7:1)	9:7 (1.3:1)	0.63
		BMI (kg/m^2)	25.6 (4.5)	29.5 (8.5)	0.009
		Height (m)	1.63 (0.10)	1.70 (0.11)	0.46
		T/Ø	1.07 (0.17)	1.00 (0.00)	<0.001
		No. of measurements*	10 (7–12)	13 (12–13)	<0.001
		Storage time (months)	1.6 (0.9)	13.6 (15.8)	<0.001

T/Ø = test location.

The means (SD) are shown and *t* tests performed for normally distributed variables; the medians (IQR) are shown and Mann-Whitney *U* tests performed for not normally distributed variables (*).

RPI of the inferomedial femoral neck

When indenting only in the inferomedial region (within ±10°), the indentation depth was still observed to be higher in the osteoporotic fractured group, as when indenting circumferentially around the femoral neck (Fig. 2*C*). However, the difference between the two groups was less pronounced (indentation depth 8.9% to 15.2% greater in the fracture group compared with 24.0% to 31.9% for circumferential measurements) and was only statistically significant in terms of TID and CID (Fig. 2*C* and Table 2 [IM]) and not IDI. Furthermore, the discriminative ability of RPI in this location was reduced (ROC = 0.60 to 0.73), can add less effectively to existing factors (RPI + CRF, AUC = 0.77 to 0.93 or CRF then RPI, AUC = 0.52 to 0.88), and when this comparison was adjusted (model 1 or model 2), the differences were found to be no longer statistically different ($p > 0.05$).

Discussion

We have demonstrated that bone from osteoporotic fracture patients is less resistant to indentation than bone from controls with no documented bone disease in one of the largest studies to date and consistent with previous findings but at a key fragility fracture site. Furthermore, these RPI differences remained after adjustment for age, sex, BMI, and height and appear distinct from current clinical measures with potential to supplement BMD and FRAX.

Limitations

Although our study is one of the largest to investigate RPI, the numbers are still relatively modest, and there are a number of limitations that should be considered in the interpretation of our results. First, the cross-sectional design does not permit the conclusion that RPI measures will predict incident fracture. It also does not allow a proper investigation of the role of RPI alongside FRAX, which is designed to identify those individuals who would benefit from treatment for osteoporosis, rather than to identify individuals who have fractured. Second, although the results do confirm clinical differences measured at the tibia,[5,22] and there is a known relationship between peripheral measures and hip fracture risk,[3,23] we have also shown that RPI varies along the femur.[16] Furthermore, the femoral neck measurements appeared to be site specific, and discrimination was less robust for those in the inferomedial region as discussed by Coutts and colleagues in terms of density.[19] This is perhaps because of RPI properties, similar to porosity[17] and thickness,[16,18] being protected through stance loading, whereas

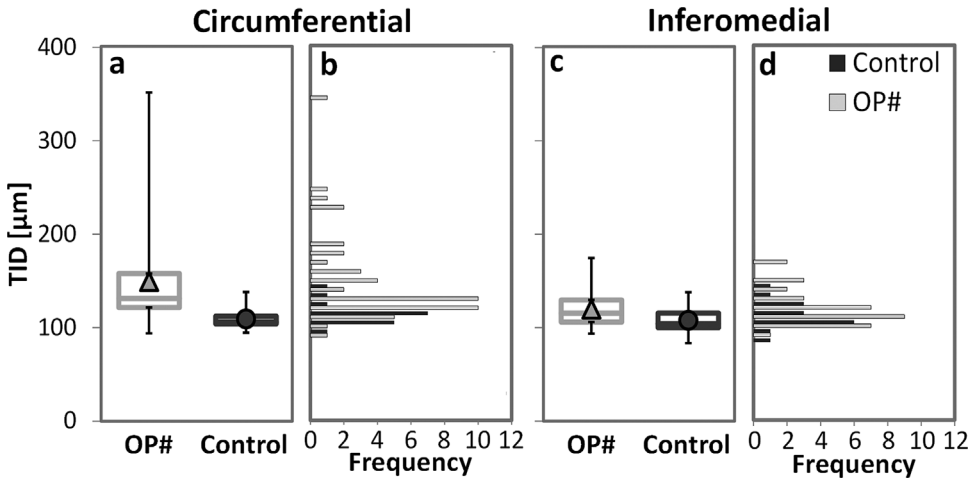

Fig. 2. Comparison between the TID in osteoporotic fracture (OP) and control group measured (*A*) in the circumferential test location (13 measurements at 20° spacing, excluding the superolateral quadrant) and (*B*) in the inferomedial test location (15 measurements with ±10° of the inferomedial point). Box and whisker plots (*A, C*) indicate range, interquartile range, median, and mean. The histograms are also plotted (*B, D*) next to these summary box-plots for each group (fracture cases as triangles and control as circles) to indicate the distribution and degree of overlap.

fractures initiate superolaterally in a fall.[24] This site dependence, as well as use of the preclinical (Biodent) device, makes it unclear how the clinically determined tibial measurements (using the Osteoprobe) relate to the clinically inaccessible femoral neck and necessitates this comparison to be made to further develop RPI. The similarities between our findings at the femoral neck and previous publications at the tibia in terms of fracture/non-fracture comparison and ROC values are in contrast to the site dependence of RPI and heterogeneity of the skeleton. This contrast implies both a degree of dependence and independence between RPI at the tibia and femoral neck. This speculation is supported by the recent publication by Abraham and colleagues,[8] which indicated tibial IDI had some yet partially independent correlation with femoral neck strength ($r = -0.478$), supporting this site dependence and need for direct comparison between the two locations. Third, owing to ethical restrictions, it was only possible to source non-disease control bone from cadaveric donors, and thus the possibility of

Table 2. Comparison Between the Osteoporotic Fracture (OP) and Control (C) Group in Terms of the RPI Parameters (TID, IDI, and CID) in the Circumferential (Circ.) and Inferomedial (IM) Locations and Clinical Factors (Femoral Neck BMD, Hip FRAX Score, and Hip FRAX Score Inclusive of BMD)

RPI		OP:C	OP	Control	*p*0	*p*1	*p*2
Circ.	TID (µm)*	46:16	131.3 (121.3–158.2)	105.9 (103.6–113.0)	<0.01		0.051
	IDI (µm)		20.1 (3.9)	16.1 (3.3)			0.30
	CID (µm)		9.5 (2.4)	7.2 (0.7)			0.081
IM	TID (µm)	34:16	119.8 (20.9)	107.8 (13.6)	0.041	>0.05	
	IDI (µm)		15.9 (3.7)	14.6 (3.5)	0.22		
	CID (µmv		7.6 (1.5)	6.6 (0.7)	0.014		
Clinical risk factors					*p*	Sens.	Spec.
FN BMD (g/cm^2)*		19:16	0.60 (0.54–0.66)	1.15 (0.77–1.36)	<0.01	37	94
FRAX (%)*		37:16	11.0 (4.6–17.0)	1.3 (0.7–3.8)		14	100
FRAX (BMD) (%)*		19:16	7.8 (4.5–17.0)	0.1 (0.0–1.3)		40	94

The means (SD) are shown and *t* tests performed for normally distributed variables; the medians (IQR) are shown and Mann-Whitney *U* tests performed for not normally distributed variables (*). For the RPI parameters, the *p* value is shown for the unadjusted model (*p*0), the minimally adjusted model 1 (*p*1), and the further adjusted model 2 (*p*2). For the clinical factors, the *p* value is shown as well as the sensitivity (Sens.) and specificity (Spec.) based on the existing clinical thresholds.

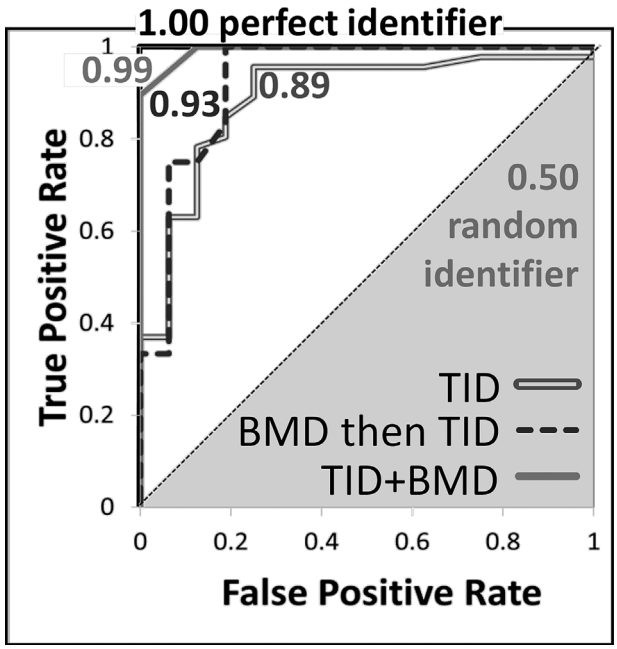

Fig. 3. Example receiver-operating characteristic (ROC) curve for TID alone and combined with BMD either by normalization and summation (TID + BMD) or in the subset not defined by existing clinical thresholds (BMD then TID).

undocumented bone abnormalities remains; the controls tended to be younger (to a greater extent than previous publications), and although RPI was not strongly associated with age, and indeed adjustment for age did not remove the differences in RPI measures between fracture cases and controls, there is still the possibility of residual confounding. Fourth, the presence of the fracture also limited indentation, necessitating a testing location likely to reduce the RPI depth in this group: ie, more distal (negative correlation, $r = -0.31$ to -0.38, $p < 0.05$) and missing superolateral measurements associated with higher indentation depth[16] in 40% of samples. Finally, freezing has previously been shown to have minimal effect on bone properties such as stiffness or strength[25,26] or RPI,[27] which also applies to this study ($r = -0.11$ to 0.12 and $p > 0.05$). Additionally, in this study, the fracture samples were carefully stored (shorter period before freezing, shorter storage duration, lower temperature and better hydrated). Therefore, if storage, location, and number of measurements are to have any confounding effect, it is likely that the difference between fractured and control group are more pronounced than observed here and, regardless, CID and TID differences remained close to significance after adjustment for these parameters.

Table 3. The Receiver-Operating Characteristic (ROC) Area Under the Curve (AUC)

	OP:C	TID	IDI	CID
RPI	46:16	0.89	0.79	0.88
RPI + CRF				
BMD	19:16	0.99	0.92	0.98
FRAX	37:16	0.95	0.88	0.93
FRAX (including BMD)	23:16	0.96	0.94	0.95
CRF then RPI				
BMD	12:16	0.93	0.82	0.94
FRAX	32:16	0.88	0.78	0.85
FRAX (including. BMD)	14:16	0.90	0.82	0.91

OP:C indicates the number of samples in the osteoporotic fractured and control groups. The ROC AUC is calculated for RPI alone and in combination with clinical risk factors (CRF – BMD or FRAX) either by summation of normalized RPI and CRF to give a combined score (CRF + RPI) or by first excluding those identified by existing clinical thresholds then performing ROC analyses with RPI in the currently undetected subset (CRF then RPI).

Comparison with previous findings

Four previous studies have explored the role of RPI in discriminating fracture cases from controls. In agreement with the in vivo measurements at the tibia by Diez-Perez and colleagues,[5] Güerri -Fernandez and colleagues,[22] and, using the Osteoprobe device, Malgo and colleagues[7] (25% to 47% greater indentation depth, 4.5% lower BMS[7]), in our study, the indentation depth was significantly greater in cortical bone tissue from individuals who had sustained an osteoporotic fracture than in those who had not. The study of Milovanovic and colleagues[28] lacked statistical power to formally demonstrate definite differences in RPI measures at the femoral neck by fracture status (5 fractures and 4 controls), although documented a potential increased indentation depth (3% to 12% higher) in fracture cases. In the present study, with larger numbers (46 fractures and 16 controls), we demonstrate a statistically significant difference at the femoral neck.

Relationship with clinical risk factors and BMD

The correlation between RPI and FRAX or BMD is generally not significant and only explains a small proportion of the RPI measures whether considering the presented Biodent system or measures of BMS in the literature using the Osteoprobe device. Although Malgo and colleagues[7] present significant negative correlation between BMS and FRAX, the authors also indicate a large independent component of RPI ($r = -0.36$ to -0.43 for BMS, $p < 0.001$), though this study utilizes the somewhat different Osteoprobe device. Within FRAX, there is only a partial correlation with age, supported by the variable but not all-encompassing ($|r_{max}| = 0.54$) correlation within the literature[5,7,10] and a non-significant or partial relationship with femoral neck BMD supporting the previous findings by Diez-Perez and colleagues[5] and Abraham and colleagues[8] with the Biodent device and Malgo and colleagues[7] with the Osteoprobe. Further risk factors that are not incorporated within FRAX (Supplemental Table S1) also only explain a small proportion of RPI, although some of these relationships have small numbers (eg, pQCT on 8 participants) and may be underpowered. Taken together, this gives the impression that RPI is measuring an element of fracture risk distinct from those that are typically assessed clinically (eg, a material property such as fracture toughness or strength[5,8–12]). As a result, high AUCs from ROC analyses indicated that RPI could supplement BMD and FRAX either by summation to create a combined score or by analyzing only those not currently identified by FRAX or BMD thresholds. In this study, BMD followed by RPI is the most effective combination. Indeed, based on this cohort, an arbitrary RPI threshold could be hypothesized (eg, a TID > 115 μm or a CID > 8 μm, these values should not be extrapolated to other studies)

to speculate a sensitivity up to 100% (for an 80% specificity) when applied alongside the BMD threshold for osteoporosis (*T*-score < -2.5). Although these thresholds are specific to our study (including this specific anatomical location, device, and test setup), Malgo and colleagues[7] also indicated, at the tibia using the clinical Osteoprobe device, that in an osteopenic subset, in terms of BMS, fracture cases were still significantly different from controls. Performing the same analysis, our study further supports the adjunctive power of RPI[7] with observations that the indentation depth remains higher in osteopenic fracture cases at the femoral neck ($p < 0.01$) and extends this to apply to the NOGG threshold for FRAX[20,21,29] ($p < 0.05$). The supplementary nature of BMD to RPI has recently been supported by Abraham and colleagues[8] with the two combined giving improved correlation with femoral neck strength. Finally, greater indentation depth may not universally indicate increased fracture risk and, despite relationships to microcracking and material properties,[5,9–12] the mechanisms involved are not fully elucidated. Consequently, a handful of murine models have implied that some conditions relating to elevated fracture risk (eg, hyperlipidemia,[30] osteogenesis imperfecta,[31] and chronic kidney disease[32]) may show increased indentation distance, whereas others (eg, diabetes[33]) may reduce indentation depth. The applicability of these diseases and other conditions linked to fracture risk (eg, rheumatoid arthritis, hyperparathyroidism, or hyperthyroidism) or being protective of osteoporosis (eg, osteoarthritis) and their translation to human bone is currently unclear and requires investigation for interpretation of RPI.

This study has demonstrated increased indentation depth in femoral neck bone samples collected at operation from patients with low-trauma hip fracture compared with non-fracture controls. Notwithstanding limitations of the cross-sectional design and preclinical technique, the documented ability of RPI to distinguish fracture patients from non-fracture controls, independent of BMD and clinical risk factors, provides further evidence in support of direct assessment of bone biomechanical properties in fracture risk assessment. Direct comparison of clinical assessment at the tibia and ex vivo measures at the femoral neck, together with exploration of the technique in prospective cohorts with fracture outcomes, is now required.

Disclosures

All authors state that they have no conflicts of interest.

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