New Tools for Assessing Fracture Risk

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1.0 Background

Bone fractures are a widespread and costly problem (1). A meta analysis of clinical studies concluded that people with type 2 diabetes mellitus (T2D) are at a higher-than-expected risk of a hip fracture than those who do not have the disease (2). Costs associated with treating osteoporotic fractures alone exceed $17.5 billion annually in the US (3), and the elderly who sustain a hip fracture are 4.6 (men) and 2.8 (women) times more likely to die within a year (4). Despite the economic burden to society and poor quality of life that fractures impose, the cause for the disproportionate increase in fracture risk with aging and T2D is unknown.

Beyond bone strength and aBMD to assess fracture risk

Traditionally, osteoporosis is viewed as a problem of low bone density causing reduced bone strength. However, there is a well-known disproportionate increase in fracture risk relative to the age-related decrease in bone density (5-8), which is commonly attributed to certain limitations in the clinical measurement of aBMD. Namely, DXA is a projection method that does not discern the relative contribution of macro-structure, micro-architecture, collagen integrity, or porosity to fracture resistance. This is one reason why the NIH held a conference on bone quality defining it as "the sum total of characteristics of the bone that influence the *bone's resistance to fracture*" (9) and the reason why the World Health Organization developed FRAX, an on-line fracture risk calculator using risk factors as input (10). To date, there is no definitive bone quality measurement that improves the assessment of fracture risk, and FRAX still underestimates risk for certain prevalent diseases like diabetes (11). Thus, **what would be useful to improving clinical care is a diagnostic assessment that actually relates to the mechanism by which aging and diabetes lowers fracture resistance**.

Reference Point Indentation (RPI) measurements discriminate fracture patients from non-fracture patients

In a hospital in Barcelona, RPI was performed using the BioDent instrument on the tibia mid-shaft of 27 women (79.1 ± 7.8 years) recovering from an osteoporotic fracture and 8 controls with a similar age range (83.2 ± 5.3 years) (12). The indentation distance increase (IDI) for the fracture patients was 47% greater (i.e., more susceptible to damage formation) than for the non-fracture patients (p=0.008). In a follow-up study, the same research team performed similar RPI tests for 4 groups of patients: 20 with no history of a fracture

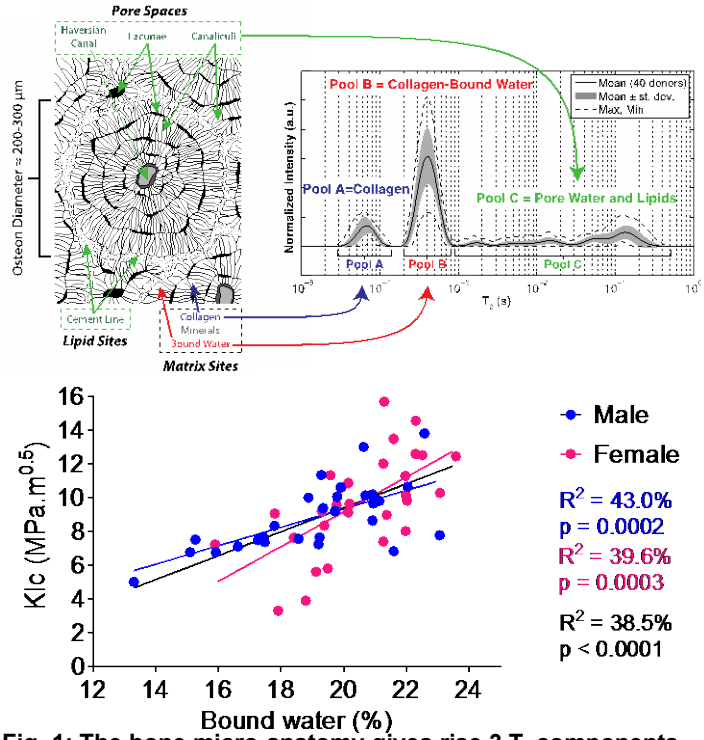

Fig. 1: The bone micro-anatomy gives rise 3 T_2 components when analyzed by ^{1}H NMR spectroscopy. One of these components, bound water correlates with the fracture toughness (K_{Ic}) of bone.

or bisphosphonate (BP) use, 38 with typical hip fractures, 6 with atypical fractures (below lesser trochanter), and 6 on BP for 5 to 12 years (but no fracture). Total indentation distance (TID) and IDI were significantly higher (worse) for the fracture cases than for the non-fracture cases (no difference between typical and atypical). The proposed project aims to assess a new, hand-held RPI instrument (OsteoProbe) for its ability to discriminate osteoporotic bone from normal bone in comparison to the clinical gold standard DXA. Although the bone measurement from OsteoProbe at the tibia mid-shaft was found to be different between women with and without type 2 diabetes (13), there is currently no evidence that it is any better than DXA in predicting fracture risk.

Other New Assessment Tools for Fracture Resistance Assessment

Current efforts in the labs of the Co-PI Dr. Nyman and his collaborators to improve fracture prediction include Raman spectroscopy (RS) (14-15), **(NMR) relaxometry** (i.e., quantitative, sub-voxel MRI) (16-17), and **reference point indentation (RPI)**. All these techniques can potentially be translated to the clinical assessment of bone, but NMR/MRI (18,19) and RPI (12,20) are closest to providing clinically useful information about bones. Moreover, the NMR technique can be combined with other MRI approaches that provide structural and architectural parameters of bone (21).

After establishing the biophysical origins of proton signals within human cortical bone using NMR relaxometry (Fig. 1) (17), we reported that bound water can explain the age-related decrease in the material strength of human cortical bone (r^2=62%, $p<0.001$) (22) and that loosely bound water detected by NMR contributes to bone toughness (16). Perhaps more relevant to diabetes and aging effects on bone, we found that bound water also correlates with the ability of bone to resist crack propagation (Fig. 1). We hypothesize that increasing pore water (intra-cortical porosity) and decreasing bound water (surrogate of 'matrix quality') makes bone susceptible to fracture.

BioDent-RPI is a relatively new analytical technique that provides properties related to the ability of tissue to resist micro-indentation (a micron-size probe tip penetrates bone tissue over 10-20 cycles in force control). As such, the best way to quantify resistance to indentation is still an unresolved issue, and differences in RPI properties are likely context or disease dependent. Thus, we performed a series of experiments to show that RPI i) is sensitive to bone tissue organization (anisotropy), ii) provides significant correlations, albeit weak, with apparent bone fracture toughness (r^2=24%, $p<0.05$), and iii) resistance to indentation decreases with age (manuscript under review). We hypothesize that low resistance to indentation is indicative of fragile bone.

Innovation

The proposed studies are based on i) *novel view* that water, collagen, and damage are important to fracture resistance, ii) *state-of-the-art* analysis tools that go beyond bone mass to quantify properties of the matrix, and iii) *unique* access to patients with clear differences in fracture resistance through an orthopaedic clinical at VUMC that fixes 100 distal radius fractures per year. These studies have the potential to i) *shift the paradigm* of bone health to one that includes characteristics of the bone matrix and its contribution to microdamage resistance, and ii) *transform* the manner in which clinical diagnostic methods for bone health are developed, from solely X-ray based to include MRI-based and mechanical characterization tools.

2.0 Rationale and Specific Aims

The goal of this study is to determine whether two new, non-X-ray techniques can discriminate between high-energy fractures of normal bone (trauma) and low-energy fractures (fragility) of osteoporotic bone. The current gold-standard for assessing fracture risk – areal bone mineral density (aBMD) by dual energy X-ray absorptiometry (DXA) – is not particularly effective at identifying individuals who are at risk of suffering a fracture (5, 23-25). Yet, there is a growing population of diabetics and elderly individuals prone to fractures (6, 26-28). In effect, the age-related and diabetes-related increase in fracture risk is independent of a person's aBMD (6, 7, 29, 30). These findings stress the urgency in developing diagnostic tools that can improve fracture risk prediction so that patients can be treated with the appropriate anti-fracture therapies.

Shifting from the current paradigm of using X-ray based modalities to assess fracture risk, the reference point indentation (RPI) method acquires direct measurements of 'material quality' of the bone tissue (31), while magnetic resonance imaging (MRI) assesses bound water and pore water concentrations that relate to the material properties of cortical bone (18,22). Specifically, the OseteoProbe-RPI engages the patient's bone, and upon applying an impact force, it measures the ability of the tissue to resist micro-indentation. Recently, this measurement – currently known as bone material strength (BMS) – was found to be lower in post-menopausal women with type 2 diabetes than age-matched women without diabetes (13). Our novel MRI methods utilize relaxation-selective preparations with ultra-short echo-time (UTE) acquisition to separately measuring bound and pore water concentrations on bone (19), which we postulate to reflect matrix quality and porosity (16). Despite the encouraging results from studies applying RPI and UTE-MRI to bone, there is no evidence that *a local measurement of bone material quality or bulk measurements of bound and pore water are predictive of fracture resistance at sites that are prone to fragility fractures*.

By affirming the ability of these tools to discriminate a fragility (osteoporotic) fracture from high-energy (trauma) fracture, the proposed study will provide justification for large prospective studies that i) evaluate the ability of these techniques to predict fracture, ii) assess whether these techniques are informative on how drug therapy is affecting bone, and iii) whether intra-operative RPI is useful to surgical guidance (e.g., placement of bone screws).

AIM 1: DETERMINE WHETHER INDENTATION RESISTANCE IS DIFFERENT BETWEEN PATIENTS WITH FRAGILITY FRACTURES AND THOSE WITH HIGH ENERGY FRACTURES.

Hypothesis: Local indentation resistance (BMS) of the distal one-third radius is lower for patients with a fragility wrist fracture than healthy patients with a traumatic wrist fracture.

Approach: Men and women (>18 y.o.) undergoing surgery to fix a distal radius fracture involving the metaphysis will be recruited into the study. Exclusion criteria will include known risk factors of pathological fractures (e.g., bone metastasis) and long-term or recent treatment for osteoporosis (e.g., bisphosphonate). In addition to standard-of-care, the orthopaedic surgeon will indent the cortex between the one-third distal radius (10-15 sites separated by 2 mm) and the ultradistal (UD) site with the OsteoProbe prior to stabilizing the fracture with a volar plate. The patient's arm will be secured to prevent rotation of the bone. Postoperatively, the patient's hips, spine, and contra-lateral radius will be imaged by DXA to determine aBMD following standard protocols at the Vanderbilt Clinical Research

Center (CRC). Patients will be stratified into two groups: high-energy fracture (e.g., motor vehicle crash) of *normal bone* and low-energy fracture (e.g., fall from a chair or standing height) of *osteoporotic bone*. We expect BMS to be less for the fragility fractures than for non-osteoporotic fractures. In addition, the lower BMS for the fragility fracture group will be significant when including aBMD, age, and body mass index (BMI) as covariates.

AIM 2: DETERMINE WHETHER BOUND WATER AND PORE WATER ARE DIFFERENT BETWEEN PATIENTS WITH FRAGILITY FRACTURES THAN THOSE WITH HIGH ENERGY FRACTURES.

Hypothesis: Bulk bound and pore water of the distal one-third radius will be lower and higher, respectively, for patients with fragility wrist fractures than healthy patients with a traumatic wrist fracture.

Approach: Postoperatively, patients in Aim 1 may also have their contralateral arm (distal one-third) imaged by our unique UTE-MRI technique (Philips Achieva 3T scanner) using wrist coil at the VUIIS. By including reference markers in the scan, the average concentration of bound water and pore water (mol ^{1}H per bone volume) will be quantified for a 14 mm axial segment (0.5 mm in-plane resolution). We will also image age-matched and gender-matched individuals without a history of fractures to the operative fracture patient group. We expect the fragility fracture patients to have significantly less bound water and more pore water than high-energy fracture patients and non-fracture patients, and this difference will be significant after adjusting for aBMD and BMI. In addition, bound water will be less and pore water more for the elderly fragility fracture group than for the elderly non-fracture group (also adjusting for aBMD). A group of non-operative distal radius fracture patients will also be recruited to undergo a DXA scan at the CRC and some of those patients will be asked to undergo an MRI scan at VUIIS.

3.0 Animal Studies and Previous Human Studies

PRELIMINARY STUDIES

Since we were able to successfully apply the BioDent-RPI instrument to human cortical bone, we became intrigued about the possibility of using the newer RPI instrument from Active Life Scientific, Inc., known as the OsteoProbe®. Unlike the BioDent® that indents the bone over 10 to 20 cycles at a target force of 10 N, the OsteoProbe-RPI indents the bone with one impact load reaching a target force of 40-45 N (31) and recording the indentation depth. The probe tip geometry of the micro-indenter is the same between the BioDent and the OsteoProbe, but the higher force generated by the OsteoProbe causes greater wear of the tip. Therefore, indentation depth into bone is normalized by the indentation depth into a reference block of plastic, thereby accounting for any subtle changes in tip surface geometry. The inventors called this ratio bone material strength (BMS), although this normalized indentation depth or Index is not necessarily a measure of strength. The misnomer aside, the OsteoProbe has the advantage of being a hand-held instrument. As long as the OsteoProbe tip is normal to the bone surface (**±** 10°**)**, there is enough internal spring force to properly trigger the impact force. In effect, an orthopaedic surgeon who has exposed a bone during surgery can use the OsteoProbe intra-operatively.

To examine the feasibility of indenting a patient's bone with the OsteoProbe, we hypothesized that BMS differs between the thicker, less porous tibia mid-shaft and the thinner, more porous forearm bones (proximal humerus and distal radius), but that the measurements at the tibia correlate with those acquired from forearm bones. Distal radius, tibia mid-shaft, and proximal humerus (left and right side) were acquired from 10 cadavers (half males and half females; 78 yo to 98 yo). While keeping the bones moist with phosphate buffered saline, 8 indents were performed in a row (~3 mm spacing) using the OsteoProbe-RPI (Fig. 2). BMS was the depth of the indent divided by the mean indentation depth into a PMMA standard (performed 8 times with each new tip and each donor). To determine whether BMS varied along the length of the bone, each BMS value per bone was normalized to the mean BMS among the 8 indents for each donor, and then these relative BMS values were plotted as a function of location of the indent (Fig. 2). In doing so, BMS increased moving from the thinner metaphysis (distal) to the thicker diaphysis portion (proximal) of the radius. *We will indent the cortex between the one-third distal radius (10-15 sites separated by 2 mm) and the ultradistal (UD) site.* Even though BMS varied among the anatomical locations but not between the left and right side (Fig. 3), tibia BMS directly correlated with proximal humerus BMS (avg of 8: r=0.69, p=0.027). Tibia BMS and distal radius BMS did not correlate (p=0.19). *We will indent distal radius before fracture fixation to determine whether BMS differs between fragility fracture and high-energy fracture cases.* Interestingly, BMS correlated with intra-cortical porosity as determined by high-resolution, micro-computed tomography (6 µm), especially for the distal radius (Fig. 4). This raises the intriguing possibility that the OsteoProbe-RPI is sensitive to micro-structure and tissue quality, two determinants of fracture resistance. Even though some of the bones had a thin cortex, the indent did not punch through the cortex (Fig. 4).

To translate and evaluate an *in-vivo* MRI protocol for

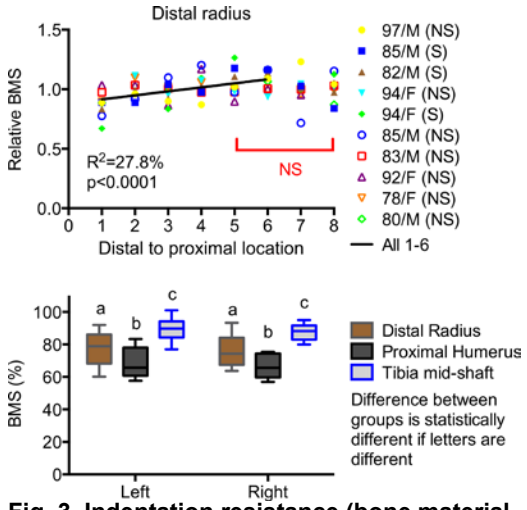

Fig. 3. Indentation resistance (bone material strength) increased from thinner metaphysis to thicker diaphysis of the distal radius. BMS varied among the different bones.

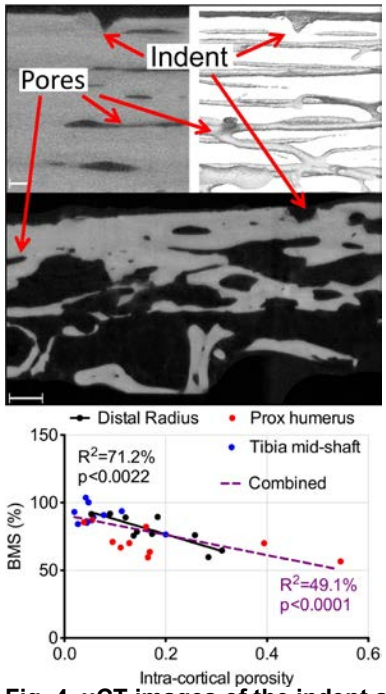

Fig. 4. µCT images of the indent and pores for the thick cortex of the tibia med-shaft (above) and thin cortex of the radius (middle). BMS is negatively related to porosity.

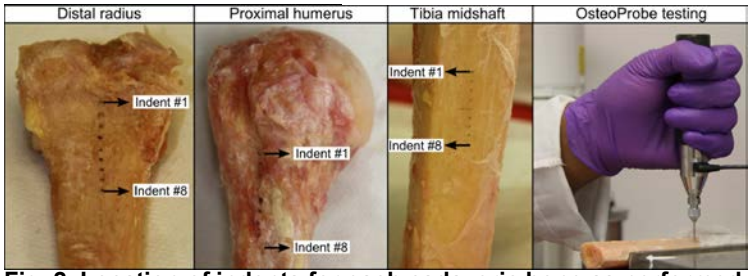

Fig. 2. Location of indents for each cadaveric bone as performed by the hand-held OsteoProbe-RPI (Right).

quantitative mapping of bound and pore water concentrations in cortical bone, UTE imaging sequences (19) were implemented on a clinical 3T scanner. Images of the lower leg and wrist were acquired on three subjects (mean age 33, M/F 1/2) to generate bound and pore water concentration maps of the tibia and radius (Fig. 5). Inter-scan variability in bound water maps ranged from 3-13% (mean 7%) in the tibia and 1-23 % (mean 10%) in the radius. Pore water maps showed inter-scan variability ranging from 5-18% (mean 10%) in the tibia and 8-27% (mean 17%) in the radius. With the addition of a new wrist coil for the 3T scanner, we anticipate a reduction in inter-scan variability by 2-4x.

4.0 Inclusion/Exclusion Criteria

Inclusion/exclusion criteria for patients with a high-energy or fragility fracture requiring operative fixation (Arm 1)

Number of patients in Arm#1= 60 patients

Inclusion criteria:

1. Patients who are 18 years of age or older. This age range accounts for 60% of all distal radius fractures seen at Vanderbilt University Medical Center.
2. Patients who have sustained a low or high energy distal radius fracture that involves the metaphysis and requires open reduction internal fixation using volar plating.
3. English speaking due to feasibility of employing study personnel to deliver and assess study intervention.

Exclusion criteria:

1. Patients who have known risk factors of pathologic fractures (e.g. bone metastasis)
2. Patients who have received treatment for osteoporosis (e.g. bisphosphonate) within the last 5 years or whose treatment lasted longer than 5 years
3. Patients who have Type 1 diabetes
4. Patients who have other bone disease (e.g., osteogenesis imperfecta, Paget's disease, thyroid disease, Vitamin D deficiency, hyperparathyroidism)
5. Patients that have a history of cancer, abnormal serum calcium, or chronic steroid use
6. Patients that would not be able to have a DXA scan (weight >350lbs, hardware in hips, patients that have lap band device)
7. Patients who are pregnant or who think they may be pregnant
8. Patients that have a medical contraindication to MRI (if patients are undergoing a study MRI)
9. Patients who have concurrent, bilateral upper extremity fractures where hardware or casting may affect study scan measurements
10. Patients who have distal radial shaft fractures

Exclusion criteria 1-5 are included because the goal of the study is to demonstrate whether new bone measurements differentiate normal bone from fragile bone. These exclusion criteria eliminate confounding factors that affect fracture resistance of bone in ways that are independent of osteoporosis. Exclusion criteria 6-8 are practical since DXA/MRI scans cannot accommodate all people.

Inclusion/exclusion criteria for patients with no fracture (Arm 2)

Number of patients in Arm#2= 40 patients

Inclusion criteria:

1. Patients who are 18 years of age or older.
2. Patients who have no history of fracture or family history of pathologic fracture
3. English speaking due to feasibility of employing study personnel to deliver and assess study intervention.

Exclusion criteria:

1. Patients who have received treatment for osteoporosis (e.g. bisphosphonate) within the last 5 years or whose treatment lasted longer than 5 years
2. Patients who have Type 1 diabetes
3. Patients who have other bone disease (e.g., osteogenesis imperfecta, Paget's disease, thyroid disease, Vitamin D deficiency, hyperparathyroidism).
4. Patients that have a history of cancer, abnormal serum calcium, or chronic steroid use.
5. Patients that would not be able to have a DXA scan (weight >350lbs, hardware in hips, patients that have lap band device)
6. Patients who are pregnant or who think they may be pregnant.
7. Patients that have a medical contraindication to MRI.

Exclusion criteria 1-5 are included because the goal of the study is to demonstrate whether new bone measurements differentiate normal bone from fragile bone. These exclusion criteria eliminate confounding factors that affect fracture resistance of bone in ways that are independent of osteoporosis. Exclusion criteria 6- 8 are practical since DXA/MRI scans cannot accommodate all people.

<u>Inclusion/exclusion criteria for patients with a high-energy or fragility fracture requiring nonoperative treatment (Arm 3)</u>

Number of patients in Arm#3= up to 10 patients

Inclusion criteria:

1. Patients who are 18 years of age or older. This age range accounts for 60% of all distal radius fractures seen at Vanderbilt University Medical Center.
2. Patients who have sustained a low or high energy distal radius fracture that requires nonoperative treatment
3. English speaking due to feasibility of employing study personnel to deliver and assess study intervention.

Exclusion criteria:

1. Patients who have known risk factors of pathologic fractures (e.g. bone metastasis)
2. Patients who have received treatment for osteoporosis (e.g. bisphosphonate) within the last 5 years or whose treatment lasted longer than 5 years
3. Patients who have Type 1 diabetes
4. Patients who have other bone disease (e.g., osteogenesis imperfecta, Paget's disease, thyroid disease, Vitamin D deficiency, hyperparathyroidism)
5. Patients that have a history of cancer, abnormal serum calcium, or chronic steroid use
6. Patients that would not be able to have a DXA scan (weight >350lbs, hardware in hips, patients that have lap band device)
7. Patients who are pregnant or who think they may be pregnant

8. Patients that have a medical contraindication to MRI (if patients are undergoing a study MRI)
9. Patients who have concurrent, bilateral upper extremity fractures where hardware or casting may affect study scan measurements

Exclusion criteria 1-5 are included because the goal of the study is to demonstrate whether new bone measurements differentiate normal bone from fragile bone. These exclusion criteria eliminate confounding factors that affect fracture resistance of bone in ways that are independent of osteoporosis. Exclusion criteria 6-8 are practical since DXA/MRI scans cannot accommodate all people.

This study will proceed until all eligible patients have been enrolled and have completed their follow-up.

Randomization
There is no randomization aspect to this study.

5.0 Enrollment/Randomization

In this prospective, clinical trial we will enroll 60 patients set to undergo volar plate fixation for a distal radius fracture and up to 10 patients receiving nonoperative treatment for a distal radius fracture. Operative patients will be stratified into two groups: 30 high-energy fractures of otherwise normal bone (e.g. motor vehicle crash) and 30 low-energy fractures of osteoporotic bone (e.g. fall from a chair or standing height). Patient study eligibility will be determined through a weekly assessment of the electronic clinic schedule, patient medical records, consultation visits, and by Vanderbilt Hand & Upper Extremity surgeons during preoperative/initial treatment clinical visits. If the patient is determined to be eligible for the study the treating physician (attending, resident, or fellow) will explain the study to the patient and ask if they would like to participate.

Physical Consent Form Process:
If the patient agrees they will be asked to review/sign an informed consent document by the treating physician or other key study personnel. During this time the patient will be given the opportunity to ask any questions they may have about the study. If the patient signs the consent form, the investigator or key study personnel will provide them with a copy of the signed form and a study contact number to call if they have any further questions. Consent will take place in the confines of a private area (e.g. clinic room).

REDCap-based Electronic Consent Form:
The patient consent process may be conducted using a Redcap-based electronic consent form. The consent form has been developed in REDCap, a secure, web-based, HIPAA-compliant, data collection platform with a user management system allowing project owners to grant and control varying levels of access to data collection instruments and data (e.g. read only, de-identified data views) for other users. Potential participants may participate in the consent process by: being approached in-person at VUMC and accessing the REDCap survey via iPad or other portable electronic device. During the in-person consent process, patients will be consented by a member of the key study personnel. Patient signatures will be obtained using a typed or written-signature (via stylus/cursor, etc.). Upon completion of the consent, patients will be provided with a copy of their version

of the consent document by printing a pdf copy of the consent form in clinic/hospital or by providing a home address for mailing of a hard copy of the consent.

In addition to the 60 operative and 10 non-operative distal radius fracture patients, we will enroll 40 healthy volunteers who have no history of a fracture. The age and gender of these individuals will be selected to match the demographics of the distal radius fracture patients requiring operative fixation. These individuals will be recruited from the metropolitan Nashville community. The means of advertising will include flyers placed in prominent campus and community locations, VICTR research notifications distribution list and ResearchMatch (Appendix E), and word-of-mouth. A potential subject responding to one of these notifications would call key study personnel. Initial screening will take place over the phone. Key study personnel will explain the study to the volunteer and give them an opportunity to ask any questions they may have. If the subject is still interested and eligible they will be scheduled for an enrollment visit, a DXA scan and an MRI. At the enrollment visit, key study personnel will again explain the study to the study volunteer and ask if they would like to participate. If the study volunteer agrees they will be asked to review/sign an informed consent document. During this time the volunteer will be given another opportunity to ask any questions they may have about the study. If the volunteer signs the consent form, key study personnel will provide them with a copy of the signed form. Consent will take place in the confines of a private area (e.g. meeting room).

6.0 Study Procedures

Arm 1- Patients with a high-energy or fragility fracture requiring operative fixation

Enrollment/Initial Visit- Study patient eligibility will be determined through a weekly assessment of the electronic clinic schedule, patient medical records, consultation visits, and by Vanderbilt Hand & Upper Extremity surgeons during preoperative clinical visits. The aforementioned inclusion/exclusion criteria will be used to determine eligibility. All patients who meet the general inclusion/exclusion criteria for Arm 1 and have elected to undergo routine volar plate fixation for their distal radius fracture will be asked to participate in the study by key study personnel. If the patient agrees to participate they will be asked to review/sign an informed consent document. Patients will also be asked to complete the following surveys: DASH (Disability of Arm, Shoulder, and Hand) and PRWE (Patient Rated Wrist Evaluation). Patients enrolled electronically may have an opportunity to complete the DASH and PRWE surveys online using Redcap.

Operative Visit- Patients will undergo a routine volar plate fixation for their distal radius fracture. Once the patient has been anesthetized and prior to stabilizing the fracture with a volar plate, the patient's arm will be secured and the orthopaedic surgeon will indent the area between the one-third distal radius and the ultradistal (UD) at 10 to 15 locations (~2 mm apart) using the OsteoProbe-RPI. The indent size is approximately ~300 μm in diameter and ~300 μm in depth.

DXA/MRI Visit - Postoperatively, the patient will visit the Vanderbilt Clinical Research Center (CRC) to undergo a DXA scan of their hips, spine, and contralateral radius. In addition, the patients will undergo an MRI of their contralateral radius at the VUIIS Human Imaging Core (which is near the CRC). We will attempt to schedule this MRI on the same day that they have their DXA scan scheduled. All

imaging will be performed within FDA safety guidelines. All female participants of child-bearing potential will be asked to undergo a serum pregnancy test at the CRC prior to the DXA scan.

Postoperative Visit- Patients will be seen postoperatively in the clinic based on standard of care guidelines. This typically corresponds to visits at 8-10 days, 3 weeks, 6 weeks, and 12 weeks, although these are not mandated dates. During these routine postoperative clinic visits the following will be recorded for research purposes: bilateral grip strength (12 week), pinch strength (12 week), and range of motion (6 & 12 weeks) measurements. In addition, patients will also be asked to complete the following surveys: DASH and PWRE (3, 6, & 12 weeks).

Osteoprobe Sterilization

Each probe tip will be new and sterilized. The PMMA block will be disinfected following standard sterilization procedures. All patient bone indentations will be taken using the Osteoprobe and then the PMMA block will be indented. The Osteoprobe will not be used on the patient following indentation of the PMMA block. The RPI instrument will be covered with a sterilized sleeve.

Arm 2- Patients with no fracture (Healthy Volunteers)

Enrollment Visit- The aforementioned inclusion/exclusion criteria will be used to determine eligibility for healthy volunteers. All volunteers who report to Vanderbilt Hand Center to take part in this study will be asked to review/sign an informed consent document. Healthy study participants will also be asked to complete a DASH questionnaire.

DXA/MRI Visit- Healthy study participants will visit the Vanderbilt Clinical Research Center (CRC) to undergo a DXA scan of their hips, spine, and forearm (side chosen at random). In addition, the participants will undergo an MRI of the same forearm scanned by DXA at the VUIIS Human Imaging Core (which is near the CRC). All imaging will be performed within FDA safety guidelines. All female participants of child-bearing potential will be asked to undergo a serum pregnancy test at the CRC prior to the DXA scan.

Arm 3- Patients with a high-energy or fragility fracture requiring non-operative treatment

Enrollment/Initial Visit- Study patient eligibility will be determined through a weekly assessment of the electronic clinic schedule, patient medical records, consultation visits, and by Vanderbilt Hand & Upper Extremity surgeons during initial clinical visits. The aforementioned inclusion/exclusion criteria will be used to determine eligibility. All patients who meet the general inclusion/exclusion criteria for Arm 3 and have elected to undergo nonoperative treatment for their distal radius fracture will be asked to participate in the study by key study personnel. If the patient agrees to participate they will be asked to review/sign an informed consent document. Patients will also be asked to complete the following surveys: DASH (Disability of Arm, Shoulder, and Hand) and PRWE (Patient Rated Wrist Evaluation).

DXA/MRI Visit - Patients will visit the Vanderbilt Clinical Research Center (CRC) to undergo a DXA scan of their hips, spine, and contralateral radius. In addition, the patients may undergo an MRI of their contralateral radius at the VUIIS Human Imaging Core (which is near the CRC). We will attempt to schedule this MRI on the same day that they have their DXA scan scheduled. All imaging will be performed within FDA safety guidelines. All female participants of child-bearing potential will be asked to undergo a serum pregnancy test at the CRC prior to the DXA scan.

Follow-up Visits- Patients will be seen in the clinic based on standard of care guidelines. This typically corresponds to visits at 8-10 days, 1, 2, 3, 4, 6, 8, 12, and 16 weeks post-treatment, although these are not mandated dates. During some of these routine follow-up clinic visits the following will be recorded for research purposes: bilateral grip strength (12 week), pinch strength (12 week), and range of motion (6 & 12 weeks) measurements. In addition, patients will also be asked to complete the following surveys: DASH and PWRE (3, 6, & 12 weeks).

7.0 Risks

Potential Risks:

Infection Risk (Arm 1 only):
As with any surgical procedure there is a slight risk of infection. To minimize this risk, the OsteoProbe-RPI probe tip used to indent bone will be new and sterilized. The PMMA block will be disinfected prior to surgery. All patient bone indentations will be taken using the Osteoprobe and then the PMMA block will be indented. The Osteoprobe will not be used on the patient following indentation of the PMMA block. In addition, a sterilized sleeve will cover all other portions of the OsteoProbe machine that are located in the sterile, surgical field of the operating room. During the routine surgical procedure, the patient will be anesthetized so the use of the OsteoProbe-RPI instrument should not cause any discomfort or pain to the patient. The indent is much smaller than the bone screws that will secure the fracture plate.

Radiation Risks:
All study participants will undergo a DXA scan to measure their areal bone mineral density (aBMD), which will slightly increase their radiation exposure. The amount of radiation they receive from the DXA scan will be equivalent to exposure during a flight from Nashville to Denver or one day of exposure to natural background radiation. Study participants who are pregnant will be excluded from participating in this study due to the DXA scan requirement.

MRI Risks:
Some study participants may be undergoing an MRI to measure pore and bound water. MRIs do not use ionizing radiation so there are no known harmful side-effects associated with temporary exposure to the strong magnetic field used by MRI scanners. However, risks of MRI include:

- Ferromagnetic objects brought into the room will be pulled toward the magnet.
- If a subject has implanted metal or medical devices they may experience abnormal torques or fail to function properly.
- There is a risk of tissue heating if there is excessive power deposition of

radio-frequency electromagnetic waves.

- There is a risk of peripheral nerve stimulation if gradients are switched too rapidly.
- The subjects may experience a claustrophobic reaction when in the magnet.
- There are loud banging noises with MR imaging that may be uncomfortable.

Breach of Confidentiality Risk:
Because patient data is being collected there is a slight risk of a breach of confidentiality. To reduce this risk, most study data will be maintained in the Vanderbilt REDCap database. Vanderbilt Redcap is a secure, web-based application for building and managing online databases. The data obtained and stored in Redcap will only be accessible by research personnel. All Redcap data will be de-identified prior to statistical analysis. All Osteoprobe measurements taken will be relayed from the Osteoprobe to a laptop which is connected to the instrument during the surgical case. The imported data will be displayed in an excel spreadsheet. This excel spreadsheet will be saved on a key study personnel password-protected computer(s). In addition, some data from the excel spreadsheet will be transferred to the Redcap study database. Any physical study forms (ex. consent documents, screening forms, surveys) will be kept in a locked cabinet in the Vanderbilt Hand & Upper Extremity Center Administrative Offices for 6 years following completion of the study. Following this 6 year period, all physical study forms will be disposed of in shred-it confidentiality bins provided by VUMC, and all Osteoprobe data kept in Excel spreadsheets will be permanently deleted from the key study personnel computer(s)

Inconvenience:
The time and inconvenience of participating in the DXA and MRI scans has been considered in this study. We will make our best effort to schedule the DXA/MRI visits on the same day, and if applicable (fracture patients), on the same day as a routine care visit. Patients will be reimbursed $50 following completion of their DXA scan, and if applicable, $50 following completion of their MRI scan.

Potential Benefits:

There is no direct patient benefit to participating in this study. Patients may receive a certain psychological reward knowing that they are contributing to increased medical knowledge about fracture risk testing and potential, new diagnostic tools that may improve anti-fracture therapies in future patient populations.

Importance of the knowledge to be gained:
This proposed study is intended to determine if two new, non-X-ray techniques can discriminate between high-energy fractures of normal bone (trauma-related fracture) and low-energy fragility fractures of osteoporotic bone. By affirming the ability of these new techniques to discriminate differences in bone quality, it will provide justification for larger, prospective studies that can evaluate the ability of these new techniques to predict fracture risk, assess whether these techniques are informative on how drug therapy is affecting bone, and determine whether these techniques are useful in the surgical setting. Such knowledge is important because the current gold-standard for assessing fracture risk does not accurately identify

individuals at of an osteoporotic risk. The potential importance of this knowledge outweighs the minimal risk of harm to study participants.

8.0 Reporting of Adverse Events or Unanticipated Problems involving Risk to Participants or Others

A data and safety monitoring (DSM) team will be formed for this study, which will consist of: Drs. Donald Lee and Jeffry Nyman and other researchers involved in this study. The DSM team will meet every 6 months to review study procedures, recruitment, and adverse events that occur throughout the study. In the event of an adverse event or patient complication, both Drs. Lee and Nyman will be notified by key study personnel immediately so they can determine if any changes to the protocol need to be made. The IRB will also be notified of any adverse events that may occur as a result of the patient's participation in this study. If an unexpected issue is identified with one of the study patients, the DSM team will be responsible for determining how to address, mitigate, and/or eliminate such risk from the study. In such cases, the protocol will be terminated, until an appropriate course of action is determined by the research team, in conjunction with the IRB.

9.0 Study Withdrawal/Discontinuation

Participation in this study is voluntary. If at any time a study participant wishes to be withdrawn from the study, they may do so by contacting any of the key study personnel and letting them know they withdraw their consent. The date in which the participant withdraws their consent will be noted in their study file. Any information gathered up to the point of consent withdrawal will still be used for research and reporting.

10.0 Statistical Considerations

Data will be analyzed using the statistical software package Stata (version 11.0, College Station, TX) with the assistance of Samuel K. Nwosu, Biostatistician III. Statistical comparisons will be performed using two-sided tests at the 5% significance level. Mann-Whitney test will assess whether differences in properties between groups are statistically significant. Next, general linear models (GLMs) will be used to determine whether the case explains each property after adjusting for aBMD (or T-score), BMI, and age. Using a robust analysis with GLMs, the data will be bootstrap with 500 replicates. Based on the variance from our cadaver studies and expected mean BMS for 'normal' bone as measured by Farr et al. (32), a sample size of 15 per group will provide 87% power to detect a 11.2% difference between the 2 cases at an α of 0.05. With 60 participants (15 per variable) will allow us to determine whether fracture type significantly explains BMS after adjusting for 3 covariates (aBMD, BMI, age). Note that there will be overlap in the participants between Aim 1 and Aim 2. From our limited imaging studies, we can expect bound and pore water of normal bone to be 22 mol $^{1}H/L$ and 10 mol $^{1}H/L$. A sample size of 10 per group will provide 81% power to detect a 22.8% and 51.8% difference in bound and pore water at an α of 0.05. Note that these changes are typically associated with a significant decrease in bone strength by destructive testing (18, 22). With 20 per group we can include a covariate in the regression models.

11.0 Privacy/Confidentiality Issues

During this study every attempt will be made to keep the patient's protected health information (PHI) private. To reduce this risk, most study data will be maintained in the Vanderbilt REDCap database. Vanderbilt Redcap is a secure, web-based application for building and managing online databases. The data obtained and stored in Redcap will only be accessible by research personnel. All Redcap data will be de-identified prior to statistical analysis. All Osteoprobe measurements taken will be relayed from the Osteoprobe to a laptop (which his connected to the instrument during the surgical case). The imported data will be displayed in an excel spreadsheet. This excel spreadsheet will be saved on a key study personnel password-protected computer(s). In addition, some data from the excel spreadsheet will be transferred to the Redcap study database. Any physical study forms (ex. consent documents, screening forms, surveys) will be kept in a locked cabinet in the Vanderbilt Hand & Upper Extremity Center Administrative Offices for 6 years following completion of the study. Following this 6 year period, all physical study forms will be disposed of in shred-it confidentiality bins provided by VUMC, and all Osteoprobe data kept in Excel spreadsheets will be permanently deleted from the key study personnel computer(s).

12.0 Follow-up and Record Retention

The duration of this study will last until we have enrolled and completed follow-up on all 110 patients. All study records will be kept for six years following study closure, at which time the database/excel spreadsheets will be deleted and all forms will be disposed of properly.

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