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Version Post-Print\Accepted Manuscript

Citation (published version) Whyne, C.M., Ferguson, D., Clement, A. et al. Biomechanical Properties of Metastatically Involved Osteolytic Bone. *Curr Osteoporos Rep* 18, 705–715 (2020). <https://doi.org/10.1007/s11914-020-00633-z>

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Biomechanical Properties of Metastatically Involved Osteolytic Bone

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Abstract

Purpose of Review: Skeletal metastasis involves the uncoupling of physiologic bone remodelling resulting in abnormal bone turnover and radical changes in bony architecture, density, and quality. Bone strength assessment and fracture risk prediction are critical in clinical treatment decision-making. This review focuses on bone tissue and structural mechanisms altered by osteolytic metastasis and the resulting changes to its material and mechanical behaviour.

Recent Findings: Both organic and mineral phases of bone tissue are altered by osteolytic metastatic disease, with diminished bone quality evident at multiple length-scales. The mechanical performance of bone with osteolytic lesions is influenced by a combination of tissue level and structural changes.

Summary: This review considers the effects of osteolytic metastasis on bone biomechanics demonstrating its negative impact at tissue and structural levels. Future studies need to assess the cumulative impact of cancer treatments on metastatically involved bone quality, and its utility in directing multimodal treatment planning.

Keywords: 4-6 keywords Osteolytic Metastases, Biomechanical Properties, Bone Quality, Multi-Scale, Cancer Treatment Effects

Introduction

Bone metastases frequently occur in patients with breast, lung, prostate and renal cancers [1–3]. Bone metastases can lead to skeletal related events (SREs) which include pathologic fractures, pain, compression syndromes of the nerve root or spinal cord and metabolic disturbances [4,5]. SREs increase mortality and significantly impact a patient's quality of life, physical function and health resource utilisation [6,7]. The risk of SREs is increased for all types of bone metastases which can present as osteoblastic (bone generating), osteolytic (bone destructive), or a mixture of the two [8]. Osteolytic metastases are more common and aggressive than osteoblastic metastases and have been associated with a higher incidence of pathologic fractures [9,10]. Bone strength assessment and fracture risk prediction are critically important in guiding clinical treatment decisions aimed at preventing and/or lessening the burden of SREs, particularly as bone metastasis evolves to a more chronic disease state.

A literature search was performed to evaluate the current understanding of the effects of osteolytic metastatic disease on bone biomechanics. This review focuses on the bone tissue and structural mechanisms altered by pathology and the resulting changes to material and mechanical behaviour. Moreover, it also considers research spanning from in vitro studies through preclinical analyses to clinical data.

Osteolytic Bone Quality and Biomechanics

Osteolytic bone metastases affects multiple length-scales including the whole bone, mesoscale, microscale, and nanoscale [11]. Due to bone's hierarchical structure, the mechanical testing of bone at different physical scales is useful in isolating the origin of potential factors leading to decreased bone quality seen clinically (Table 1) [12].

Bone Matrix

Bone tissue is a composite material consisting of a combination of organic and mineral phases. Collagen fibers are the major components of the organic phase and hydroxyapatite crystals account for the majority of the mineral phase. Features within the organic phase which are used to assess bone quality include the structure and organization of collagen-I fibrils and the type and amount of collagen cross-links. The size of hydroxyapatite crystal deposits, degree of carbonation of the crystals and the heterogeneity of mineral distribution are useful in describing the quality of the mineral phase.

Organic Phase

Collagen fibril organization, morphology and packing contribute to the mechanical properties of bone [13]. The rectilinear array of collagen-I fibrils has been shown to contribute to the toughness of bone [14–16]. Collagen organization has also been shown to affect modulus and hardness [17,18]. Osteolytic metastatic involvement has been shown to impact collagen organization in both trabecular and cortical bone, with increased collagen fibril disorganization associated with reduced hardness and modulus [19–22]. While modifications in collagen fibril diameter have been associated with changes in intrafibrillar cross-linking, which in turn, have been associated with changes in bone strength and post-yield properties [23–25], no significant differences have been seen between the collagen fibril diameter of osteolytic and healthy bone [19].

Type I collagen fibrils are stabilized in part by trivalent mature pyridinium cross-links: pyridinoline (pyr), deoxypyridinoline (dpyr) and pyrrole [26]. Unfolding of these collagen cross-links enables the bone to absorb extra energy without breaking [27]. Reductions in collagen cross-links have been correlated with a lowered bending strength and elastic modulus of bone [25]. Additionally, advanced glycation end products (AGEs) such as pentosidine form cross-links between collagen fibrils [28], reducing its ability to “unfold” [27]. An increase in pentosidine concentrations is associated with diminished bone ductility, toughness and post yield properties and may act as a marker for oxidative stress [29]. Human vertebrae with reduced compressive biomechanical properties have shown elevated non-enzymatic glycation and beta isomerization of type I collagen [30]. Recent studies have shown that osteolytic bone metastasis leads to an increase in pentosidine concentration, decrease in pyr and dpyr concentration and results in a bone with inferior cross-linking chemistry [19,26]. An important feature of tumour tissue is its high glycolytic flow, producing dicarbonyl glyoxals and methyl glyoxals, causing tissue inflammation. Cancer cells protect themselves against these aldehydes by producing glyoxylases, increasing the production of AGEs [31]. In addition to glyoxylases, tumour cells also overproduce hydroxylation enzymes such as prolyl hydroxylase [32] and lysyl hydroxylase [33], generating reactive oxygen species (ROS) [34]. This can impact the cross-linkages pathway through the hydroxylation of collagen chain amino acids like proline and change the biochemistry as well as the mechanical behaviour of bone. Literature has reported altered hydroxyproline-proline ratios, indicating the deterioration of the

collagen cross-link chemistry and compromise in the mechanical properties of bone with osteolytic involvement [19,26].

Mineral Phase

Numerous studies have highlighted the impact of metastatic disease on the mineral density and distribution of trabecular bone [35–38]. A preclinical study examining the impact of metastasis on the mineral phase of vertebral bone tissue reported a slight increase in the tissue mineral homogeneity of osteolytic bone [39]. This increase in localized mineral homogeneity could lead to increased bone brittleness and fracture risk [40,41]. Osteolytic bone has also been shown to reduce the tissue mineral content [39] which has a strong correlation to trabecular bone modulus and hardness [20].

Hydroxyapatite (HA) provides bone with its rigidity and changes in its chemistry can compromise the mechanical strength of the bone [42]. Previous studies have shown that metastasis can influence HA crystal composition and size, and that these alterations can influence metastasis [42,43]. He *et al.* reported a decrease in HA crystal size and quality in both tumour involved and non-tumour involved bone and suggested that the less mature HA crystal in non tumor bone may further attract more tumor cells to bone [38]. Osteolytic bone metastases have been reported to increase the carbonation of the HA lattice and decrease the lattice strain [44]. Whyne *et al.* showed that osteolytic bone metastasis leads to a higher carbonate to phosphate ratio and lower carbonate to matrix ratio [26], resulting in a bone vulnerable to fracture and susceptible to further tumour invasion. Further experiments by this group indicated that there was a reduced HA crystal width and lowered average mineral content in osteolytic bone, yielding a bone with lower modulus and hardness [39]. These changes in the crystal chemistry may be due to the overexpression of bone sialoproteins (BSP), the nucleation proteins responsible for initiating hydroxyapatite crystal mineralization [45]. BSP overexpression increases the number of nucleation sites and the number of mineral crystals, but, lowers the size of these crystals due to spatial and ion constraints. As a result, tumour involved bone has mineral crystals with smaller size, more carbonation and inferior mechanical quality [39].

Tissue-Level Mechanical Properties

The mechanical properties of bone can be measured at various length-scales using dynamic and quasi-static indentation testing. During indentation testing, tissue hardness and elastic modulus can be determined from force-displacement curves which are generated as the indenter loads and unloads the bone tissue. Microindentation (~5µm to ~200µm) and nanoindentation (~0.1µm to ~10µm) have been used to determine the hardness and modulus of bone tissue [46].

Nanoindentation has been used to characterize the hardness and modulus of metastatic lesions. A reduction in hardness and modulus has been demonstrated in metastatically involved human vertebral bone, however this study did not distinguish between the osteoblastic/osteolytic nature of the samples [35]. Other metastatic bone models have suggested reduced bone tissue modulus with breast cancer involvement [47]. In contrast, nanoindentation performed on osteolytic metastatic rat vertebrae found no significant tissue level differences between the hardness and modulus of osteolytic and healthy samples [20].

Indentation techniques have been used to determine fracture toughness, however the accuracy of these methods may be overly simplified for use in bone [48,49]. Notch tests or testing of cantilever beams have been used on cortical bone tissue to quantify fracture toughness [50,51], but such testing has not been applied to bone impacted by osteolytic metastases. Opportunities exist to determine bone fracture toughness in trabecular bone and metastatically involved bone tissue through the creation and compression of micropillars of bone tissue (i.e. via ion beam milling) [52,53]. Scratch tests could also be used to evaluate bone toughness on a micro scale [54–56]. However, no such tests have yet been performed on osteolytic metastatic bone tissue.

Microdamage

Under normal physiology, microscopic tissue damage (microdamage formation) within bone tissue serves as a stimulant for bone remodeling. However, accumulation of unrepaired microdamage can be associated with clinical fracture susceptibility, especially fractures associated with age and osteolytic bone [57]. Deterioration of quality in osteolytic bone is evident when microdamage accumulation is visualized in the tissue (i.e. through histologic staining (i.e. calcein green) or micro-CT and/or back scatter electron imaging following barium sulphate staining). Accumulated microdamage negatively impacts the mechanical strength of bone, including reductions to elastic modulus and strength [58,59]. Computational modeling has shown significantly higher stresses and strains in the damaged region of osteolytic vertebrae, which agrees with higher levels of accumulated microdamage [60].

Structural Changes

At a macro level, skeletal metastasis affects the natural resorption cycle of bone. In osteolytic metastases, bone loss can be measured as a loss of trabecular architecture and through volumetric measurements of lesions within the bone tissue [9,61–63]. Deviations from the healthy structure of bone tissue are associated with diminished mechanical integrity. Osteolytic defects have been shown to reduce the mechanical stability of bones, however accurate quantification of the impact must consider more than bone loss (lesion size) alone [64–67].

Architecture

To understand the effects of metastatic tumours on trabecular architecture, microcomputed tomography has been used to quantify morphological parameters of bone tissue in preclinical models. Stereological parameters including trabecular thickness, trabecular number, trabecular spacing and trabecular bone volume have been used to quantify changes in architecture of bone in osteolytic murine models in the femurs and the spinal column [44,68]. The architecture of osteolytic trabecular bone has been characterized by decreased trabecular number and thickness and increased trabecular spacing when compared to non-pathologic bone [20,35,37,44,69,70].

Mechanical Properties

At a macro level, mechanical testing is used to determine mechanical properties (stiffness, strength, rigidity) to better understand how disease and treatments affect mechanical stability. The mechanical strength of bones is affected by both material properties and architecture. In the context of mechanical testing, simulated defects in bone are often created to represent architectural changes caused by osteolytic disease [66,71–75]. However the lack of inclusion of tumour tissue and changes to tissue level bone material properties in these models, may limit their clinical relevance [71]. There are limited experimental studies that have tested mechanical performance of osteolytic disease in human tissue due to the inaccessibility of specimens [35,76,77]. Animal models of skeletal metastatic disease have been widely used in this context as they can incorporate both architectural and tissue material differences to better replicate osteolytic lesions when compared to simulated defects [20,44,68,69,78].

Catastrophic failure can present as varying fracture patterns depending on the loading scenario and bone specimen structure. Clinically, the presence of osteolytic metastasis in the spine has been associated with burst fracture patterns, but therapeutic treatments have lessened this type of catastrophic failure, with compression fracture patterns now more commonly observed [79–81]. Applied loading in experimental simulations (axial compression and bending, at differing loading rates) affects the resulting fracture location and type [82,83]. In long bones, three-point bending methods are commonly used in osteoporosis to evaluate changes in mechanical properties. In osteolytic metastases, due to the focal nature of the lesions, long bones are more commonly tested in torsion [73].

Vertebral Testing

Axial compressive testing has been used to characterize vertebral structural integrity in the presence of osteolytic lesions in: bone cores [35,76], whole vertebrae [69,84] and spinal motion segments [20,59,70]. All studies have found decreases in vertebral mechanical stability in the presence of metastatic disease (reduced stiffness, ultimate force, axial rigidity) [20,35,59,69,70,76,84]. Biomechanical testing performed through vertebral motion segments better represents physiological loading through the intervertebral discs and posterior elements, enabling representation of both burst and compression fracture patterns.

Researchers have observed correlations between stereological features and mechanical behaviour of bone tissue with osteolytic lesions under compressive loads. In vertebrae, relations have been identified between both stiffness and strength with bone/tissue volume ratio, as well as between ultimate force and bone mineral density [35,85]. However, bone tissue mineral density alone was not a strong indicator of macroscopic behaviour [35]. Not surprisingly, osteolytic involvement has been reported to have a reduced vertebral trabecular bone volume, axial rigidity, stiffness and failure force when compared to healthy controls [20].

Computed tomography images can be used to calculate structural rigidity measures, which are dependent on both material modulus, cross-sectional area of bone, and material distribution. Imaging based bending rigidity measures have been correlated to mechanical failure in biomechanical testing experiments [20,37,64,71,86]. Torsional biomechanical testing has been used to determine torsional rigidity and ultimate torque in vertebral bone cores with lytic lesions. Biomechanical measurements of yielding in torsion, bending and in tension were highly correlated ($R^2 > 0.9$) with the corresponding imaging derived rigidity measures, torsional, flexural, and axial rigidity, respectively. [86]. (bending, torsional, and axial) loads (bending yield moment, torsional yield moment, and yield load)

Long Bone Testing

Torsional testing is most commonly used in evaluating the mechanical behaviour of long bones with osteolytic involvement. Using this approach, femurs with osteolytic metastases have demonstrated reduced maximum torque and rigidity when compared to healthy controls in preclinical, simulated cadaveric and computer models [67,73,86]. Torque and failure energy have been seen to have moderate correlation with BMD [78].

Applied compressive loading to the femoral head is often applied to better represent anatomical loading scenarios (yielding a combination of axial compression and bending in the femur). As expected, such studies have found decreased failure loads and stiffness in samples with osteolytic disease [36,66,73,75,76]. Location of the lesion impacts the stiffness and ultimate strength measures [75,87].

Computer Modeling

Finite Element (FE) models, validated with in vitro experimental mechanical testing, have been utilized to better understand the impact of metastatic disease on bone stress and strain distribution. These models have been shown to accurately predict experimental results (apparent stiffness, ultimate strength) [59,65,69,71–73,76,77,88,89], however application to clinical data sets remains a challenge. While earlier parametric models were used to examine the impact of features, such as tumour size, location, material properties, loading, on vertebral stress and strain patterns, more recent modeling has focused on specimen specific image-based models. A notable limitation of modelling studies is neglecting the material properties of tumour and the effects of tumour or treatment on the material properties of the surrounding tissue. Validation of Finite Element Analysis (FEA) has primarily been performed against ex-vivo bone with voids created to mimic tumour shapes. Automated methods for segmentation of vertebrae and osteolytic disease from CT data facilitate generation of specimen specific FE models [90–93]. Similar approaches have been taken in segmentation of vertebrae and metastatic disease in preclinical models based on microCT and microMRI data [70].

While continuum models have been generally created from clinical imaging, microCT based models have been used to generate microFE models of vertebrae with metastatic disease. Such models have been shown to correlate strain with the generation of load induced microdamage [59,60] and suggested that microdamage may be generated at lower load strain levels in metastatically involved tissue. Current work has extended microFE modeling to represent post yield behaviour with the addition of cohesive elements – such work has demonstrated agreement with damage generated through in vitro mechanical testing in healthy and osteoblastic vertebrae [94,95]. Further this work has demonstrated that metastatic involvement affects the damage properties of bone tissue.

Clinical Biomechanics

Understanding the biomechanics of bone with metastatic involvement is important for clinical decision making, considering treatment options, monitoring response to therapy and disease progression. Many clinical factors have been identified that contribute to the instability of metastatically involved bone. The presence of existing fractures (consistent with patients without cancer) is a risk factor for future fracture [96]. In the bony spine, the malalignment of the spine (presence of scoliosis, kyphotic deformity, or vertebral subluxation or translation) also impacts risk [97]. Lung and liver tumours, older patient age and higher pain levels have also been associated with increased risk of fracture [81],[96].

Clinical scoring tools have been used to make decision making that involves combining of these many factors easier and more consistent. Scoring tools focus on determining the risk of instability in patients with skeletal metastases and can be used to triage patients deemed stable and direct interventions in those at risk of fracture or fracture progression, neurological compromise, or mechanical pain. A number of clinical scoring tools are used in the context of skeletal metastases including the Spinal Instability Neoplastic Score (SINS), Bilsky tumour grading in the spine, the Thoracolumbar Injury Classification and Severity Score (TLICS), and the Mirels' scoring system (long bones) [98–100]. SINS has been shown to have good intra (0.886) and inter (0.846) rater reproducibility [101,102], although it lacks objectivity based on quantitative metrics. Defining spinal neoplastic-related instability and the introduction of SINS have led to improved uniform reporting within the spinal neoplastic literature, however the prognostic value of SINS remains controversial [102].

CT-based structural rigidity analysis, calculated on transaxial CT images, has also been applied clinically in the identification of metastatically involved bone at risk of fracture. Prediction of the reduction in fracture risk based on CT based structural rigidity at the lesion (> 35% in axial, bending, or torsional rigidities) has been shown to be better than Mirels scoring in predicting femoral impending pathologic fracture [103]. CT-based structural rigidity outcome measures applied to the metastatic spine, have yielded 100% sensitivity, with 44% to 70% specificity, to predict

fracture risk [104]. Volumetric assessments of tumour burden and dynamic quantification of bone density changes on CT imaging have also been shown to distinguish between those patients at risk of fracture and those who remain stable [105,106]. Further complication in the bony spine is that many vertebral compression fractures will not require mechanical stabilization and remain mechanically stable, with the presence of local healing or further volumetric collapse showing a relationship with long term stability [107].

Specimen specific FEA presents an alternative to structural rigidity analysis that is potentially more sensitive to changes in tissue properties and loading conditions at the expense of greater complexity. Biomechanical models generated from FEA that quantify vertebral stability have been applied to clinical data sets with some success [106,108–110], highlighting the ability of biomechanically based guidelines to yield quantitative metrics that may aid in clinical decision making and intervention guidelines [72,76,111]. However, such approaches will require automated pipelines to realize clinical value. Current endeavours in the area of machine learning may facilitate this translation [112] with some recent work applied in the area of spinal metastases [93,113,114].

Treatment Effects

Treatments for skeletal bone metastases are designed to decrease pain, improve structural stability and mobility and control tumour growth. Treatment decisions regarding metastatic spine disease are dependent on multiple factors including clinical symptoms, the presence of neurological deficits, tumour pathology, anticipated radiosensitivity, mechanical stability, the extent of disease and the available therapeutic modalities. Treatments are often multimodal and may consist of systemic drugs (i.e. chemotherapeutics, Rank-L inhibitors, bisphosphonates), local treatments (i.e. radiotherapy, radiofrequency ablation) and structural stabilization techniques (i.e. vertebroplasty, kyphoplasty, open surgery and hardware). The impact of such treatments on bone quality are critical in understanding their impact on mechanical stability.

Radiation therapy is commonly used for treatment of metastatic bone tumours, however in the movement towards localized control with stereotactic body radiotherapy (SBRT), the incidence of post treatment fractures has increased with post SBRT vertebral compression fractures occurring in approximately 11% of patients [3,115–118]. As such, the focal impact of SBRT on bone quality [119–121] must be considered in the context of treatment planning and patient selection.

Mechanical and material properties of healthy and metastatically involved bone have also been assessed for treatments such as bisphosphonates [122–127], chemotherapeutics [128–131], photodynamic therapy (PDT) [85,132], Rank-L inhibitors [133–136], and multimodal treatment combinations [137,138]. Bisphosphonates and Rank-L inhibitors slow down bone turnover, leading to increased bone mineralization and slower osteolytic tumour growth. Clinically, bisphosphonates have been shown to reduce the risk of skeletal related events in patients with bone metastases (Relative Risk (RR)= 0.85) and Rank-L inhibitors further reducing the risk compared to bisphosphonate treatment (RR=0.78) [139]. Bisphosphonate use has been associated with osteonecrosis of the jaw, albeit rarely (0.5%) in breast cancer patients [139]. Atypical femur fracture has also been reported in patients with bone metastases undergoing antiresorptive therapies (Denosumab (anti-Rank-L antibody) and bisphosphonates) at varying levels [140]. In a recent study Ota et al reported atypical femur fracture in 7.8% of breast cancer patients with bone metastasis who received antiresorptive agents (Denosumab and/or bisphosphonates (zoledronic acid)) compared to no atypical fractures in those who did not receive antiresorptive agents [141]. The true incidence of atypical femur fracture among cancer patients on antiresorptive therapy is not currently known due to the lack of large studies [140] PDT has shown increased apparent mechanical strength (40% increase) and bone mass (45% increase) in preclinical experiments with ongoing clinical trials to verify these findings [132]. Owing to the growing arsenal of treatments, there is a need to assess the impact of cancer treatments on bone quality in osteolytic bone at various length-scales to assist with multimodal treatment planning.

Conclusion

Osteolytic bone disease and associated treatments affect the material, mechanical and structural properties of bone tissue. Bone quality assessment and fracture risk prediction are critical in determining the need for intervention and guiding multimodal treatment planning of osteolytic metastatic bone disease. Owing to the hierarchical structure of bone, it is essential to characterize the parameters that impact the mechanical integrity of metastatically involved bone at a nano, micro, meso, and whole-bone level. No tissue or structural parameter alone has been able to completely explain the mechanical performance of bone with osteolytic lesions. An increased understanding of treatment options, including their biomechanical sequelae, can hopefully reduce the incidence of pathologic fractures in the metastatically

involved skeleton, leading to reductions in health resource utilization and improvement in the quality of life for the growing number of patients with these lesions.

Compliance with Ethical Standards

Conflict of Interest: I, Cari Whyne, on behalf of all authors, declare no conflict of interest.

Human and Animal Rights and Informed Consent: This article does not contain any new studies with human or animal subjects performed by any of the authors.

Table 1: Summary of factors influencing the biomechanics of metastatically involved osteolytic bone

Factors influencing bone biomechanics	Importance	Detection method	Changes during osteolytic bone metastasis	References
Pyridinoline	Creates cross-links between collagen fibrils, stabilizing the fiber	HPLC	Decreases in bone, increases in serum and urine	[26,142]
Deoxypyridinoline				
Pentosidine	Accumulates between collagen fibrils, induces inflammation, reduces bone strength, may alter the behaviour of bone cells	HPLC	Increases	[26]
Hydroxyproline/proline ratio	Marker of oxidative stress and tissue remodeling in the tissue	HPLC, colorimetric	Increases	[26,32]
Carbonate/phosphate ratio	Higher carbonates lead to decreased lattice strain and increases susceptibility of tumour invasion	Raman microscopy	Increases	[26] , [42]
Carbonate/matrix ratio				
Bone volume,	Indicates total bone present in the vertebrae	microCT	Decreases	[85]
Trabecular thickness	Thinner trabecular bone indicates bone loss	microCT	Decreases	[85]
Trabecular number	Fewer trabeculae indicate bone loss	microCT	Decreases	[85]
Trabecular spacing	Higher spacing indicates bone loss	microCT	Increases	[85]
Bone mineral density	Lower bone mineral density indicates poor bone health	microCT	Decreases	[85]
Hydroxyapatite crystal size	Metastases decreases crystal size, attracting more tumour cells	XRD	Decreases	[39]
Microdamage	Promotes bone remodeling at normal levels, compromises bone biomechanics upon accumulation	BaSO ₄ staining followed by microCT	Increases	[59]
Presence of osteolytic lesion	Presence of defect causing alterations in structural architecture	Compression	Decreased mechanical stability (reduced stiffness, ultimate strength, axial rigidity)	[20,35,59,69,70,76,84]
Presence of osteolytic lesion	Presence of defect causing alterations in structural architecture	Bending	Decreased failure load and stiffness.	[36,66,73,75,76]
Location of lesion	Spatial location of lesion	Bending/compression	Ultimate strength and stiffness	[75,87]
Presence of osteolytic lesion	Alterations to the structural configurations of bone	Torsional testing	Reduced ultimate torque and torsional rigidity	[67,73,78,86]
Presence of osteolytic lesions	Alterations to the structural configurations of bone	Finite Element Modeling	Increases in stress/strain on bone, variations in stress/strain distribution	[59,65,69,71–73,76,77,88,89]
Collagen fibril organization	Increased collagen fibril disorganization has been associated with reduced hardness and modulus	Second Harmonic Generation Imaging	Increases disorganization	[19–22]

Collagen fibril diameter	Modifications in fibril diameter have been associated with changes in intrafibrillar cross-linking, which in turn, has been associated with changes in bone strength and post-yield properties	Transmission electron microscopy	No significant changes	[19,23–25]
Tissue mineral homogeneity	Increase in mineral homogeneity could lead to increased bone brittleness and fracture risk	Backscatter Electron Imaging	Slight increase in localized mineral homogeneity	[39–41]
Tissue mineral content	Strong positive correlation	Backscatter Electron Imaging	Decrease in tissue mineral content	[20,39]

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