Value of impact microindentation in the evaluation of bone fragility in clinical practice

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PART I:

INTRODUCTION

Chapter 1

General introduction and outline of the thesis

Determinants of Bone Strength

Bone strength is a complex concept encompassing several factors affecting the ability of bone to resist fracture and the material and structural properties of bone largely determine bone strength (1). While bone mass as evaluated by bone mineral density measurements using dual X-Ray absorptiometry (DXA) was initially relied upon as main determinant of bone strength, accumulating evidence has shown that it represents only one aspect of its multifaceted features. Structural bone characteristics such as its size, shape, and microarchitecture, as well as its material properties such as the quality of mineralization, collagen composition, and the presence of microdamage, also significantly contribute to the mechanical competence of bone not only ensuring stiffness but also flexibility. The interaction of all these components is crucial in maintaining bone strength, and alterations in one or more of these factors may compromise bone strength, leading to bone fragility and a higher risk of fracture (1, 2). Over the past two decades, the increasing understanding of the factors that may impact on bone strength and the availability of new techniques for evaluating its various properties in vivo beyond bone mass have resulted in improvement of fracture risk assessment and osteoporosis management. However, despite these significant advances, the evaluation of fracture risk still represents a significant challenge in daily clinical practice.

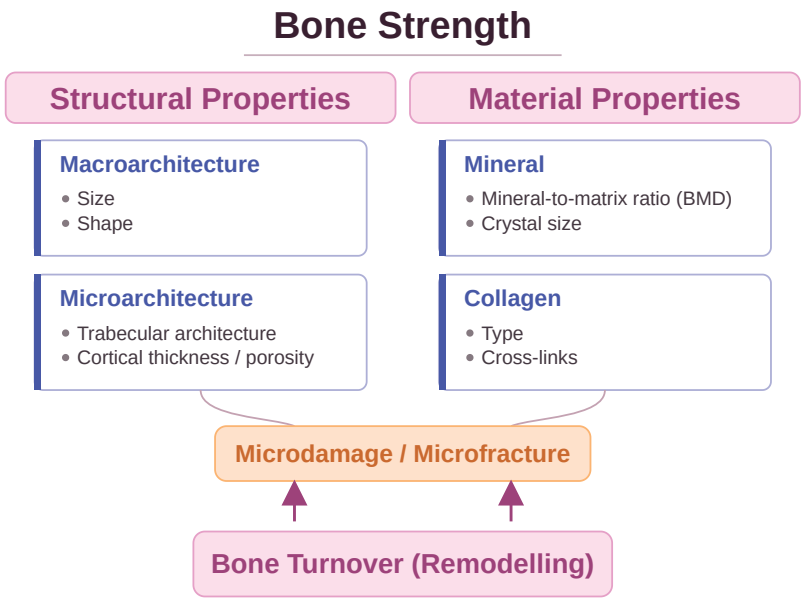


Figure 1. Determinants of bone strength. Bone strength is determined by the interaction between structural properties, material properties of the bone matrix, and bone turnover (remodelling). Disruption of any of these components may compromise the ability of bone to resist fracture.

At the macroarchitectural level, bone structure includes the overall shape (geometry) and size of bone, as well as the spatial distribution of bone mass, all of which influence mechanical competence (Figure 1) (1). At the microarchitectural level, trabecular bone consists of a network of interconnected rods and plates, predominantly located at the end of long bones and in vertebral bodies. Its architecture, defined by trabecular number, thickness and separation, plays a crucial role in absorbing energy and distributing it during mechanical loading, playing a key role in maintaining structural integrity under dynamic conditions (3). For example, vertebral bodies are rich in trabecular bone, which provides a degree of elasticity needed to withstand compressive forces during daily activities. In contrast, cortical bone, which forms the dense outer shell of the bone, provides essential stiffness and resistance to bending. Its mechanical competence is largely determined by cortical thickness and porosity. Specifically, cortical thickness contributes to the bone's ability to withstand mechanical forces, while increased porosity can weaken the bone's structural integrity (4). Cortical bone is represented by the skull, which serves as a rigid protective shell for the brain. Together, the structural features of trabecular and cortical bone demonstrate how bone architecture is optimized to meet the diverse mechanical demands of different skeletal sites. Alterations in any of these macro- and micro-architectural features, whether due to aging or disease, can significantly compromise bone strength and increase bone fragility (5, 6) .

These structural characteristics of bone interact closely with its material properties, as the composition and quality of the bone matrix determine how effectively the architectural framework can resist mechanical forces. Bone material properties include the composition and organization of the bone matrix (1) . Collagen, the main organic component of the bone matrix, provides bone with strength and flexibility, while mineralized hydroxyapatite crystals provide rigidity. The quality of collagen, which is provided by the cross-linking of its fibrils and by a balanced bone turnover, plays a key role in how well bone resists fractures (7, 8). Similarly, the quantity and spatial distribution of mineral within bone affect its stiffness and toughness. Alterations in any of these material properties, such as those resulting from ageing or metabolic bone diseases, can weaken bone's structural integrity and thus increase fracture risk.

Maintenance of normal bone remodelling, the continuous balanced process driven by bone cells: osteoblasts, osteoclasts, and osteocytes, is a critical factor in maintaining the integrity of both the structural and material properties of bone throughout life (9). The imbalance in remodelling observed in women after the menopause, and in men and women with ageing forms the basis of bone loss which produces trabecular and cortical structural damage and increased fracture risk (10). The structural damage is further exacerbated by the decreased mineralisation of bone

resulting from excessive bone resorption or inadequate bone formation or both, which alter bone's material properties, and by microdamage accumulation thus further decreasing bone strength (11, 12).

Osteoporosis and fractures: epidemiology and clinical relevance

Bone fragility and the resulting fractures are the most common clinical manifestations of osteoporosis, a term historically derived from the Greek words for 'bone' and 'pore' (''porous bones'') (13). Fractures occur when the external force applied to a bone exceeds its mechanical competence. For any given load, a bone's resistance to fracture depends not only on bone mass, but also on its structural and material properties, as described above.

Fragility fractures typically occur at sites like the hip, vertebrae, humerus, or wrist, and are traditionally defined as fractures following minor trauma that wouldn't usually cause a fracture, such as a fall from standing height or less (14). Importantly, fragility fractures are associated with significant morbidity, increased mortality, and a substantial socio-economic burden (15). Worldwide, osteoporosis and its consequent fragility fractures represent a major health challenge. Approximately one in three women and one in five men over the age of 50 will experience at least one fragility fracture in their lifetime, with an estimated 178 million new fractures globally occurring in 2019 (16-18). Due to demographic ageing, fracture numbers are projected to rise sharply. The incidence of hip fractures, which are among the most debilitating osteoporotic fractures, is thus expected to almost double by the year 2050 compared to 2018 (19). Consequently, early diagnosis and initiation of adequate treatment for osteoporosis are essential to slow down the anticipated fracture pandemic.

Causes of osteoporosis are diverse and not uncommonly multifactorial. Primary osteoporosis is mainly driven by postmenopausal hormonal changes and ageing, and fracture risk increases exponentially with age (20). Secondary osteoporosis arises from a variety of medical conditions such as endocrine, renal, liver, gastrointestinal or rheumatological diseases, and/or the treatment thereof such as glucocorticoids, which have also been shown to be independently associated with increased fracture risk due to their deleterious effects on the skeleton. Up to 40% of postmenopausal women and 60% of men with osteoporosis have been shown to also have an underlying secondary cause further contributing to their increased bone fragility (21, 22). Diagnosing bone fragility, especially in secondary osteoporosis when bone quality may be singularly or more prominently affected than bone quantity, remains a major clinical challenge (23, 24).

Since osteoporosis is a silent disease, it usually only comes to medical attention after the occurrence of a first fracture. In the Netherlands, it is recommended to evaluate for osteoporosis all patients > 50 years of age who had sustained a recent fragility fracture or one within the previous two years, in patients using glucocorticoids at high doses (≥ 7,5 mg of prednisone or its equivalent) for ≥ 3 months, and in patients aged 60 years or older in the presence of other clinical risk factors associated with a high 10-year probability of fracture as identified by a FRAX algorithm evaluation (25). Although a sustained fracture represents one of the most important risk factors for the development of a new fracture (26, 27), both the evaluation and treatment of osteoporosis following an initial fracture have been historically largely inadequate (28, 29). To address this care gap, the International Osteoporosis Foundation (IOF) launched the global initiative named "Capture the Fracture" in 2012 (30), with the main aim of improving the identification and management of patients who have recently sustained a fracture through the implementation of Fracture Liaison Services (FLS). The main goal of an FLS is to reduce the incidence of new osteoporotic fractures, improve patient outcomes, and decrease the personal, societal and socioeconomic burden associated with osteoporosis by means of early detection of fracture risk and start of treatment after a recent fracture (29, 31-33). Many of the patients and controls investigated in the studies conducted in this thesis were identified through the long-established FLS of the Leiden University Medical Center.

Given the multifactorial determinants of bone strength and the high clinical burden of fragility fractures, accurate assessment of fracture risk is of paramount importance. Since it has become abundantly clear that evaluation of bone mass alone does not fully capture other aspects of bone fragility, such as the impact of altered structural and/or material properties of bone on fracture risk, consideration of tools for evaluating both beyond the evaluation of bone mass is warranted for a more complete and accurate evaluation of true fracture risk.

Diagnostic tools for bone fragility beyond BMD DXA: current standards and limitations

Historically, the term osteoporosis was first introduced in the early nineteenth century as a pathological description of bone tissue deterioration, referring to the "cavities" observed in the bones of affected patients (13). In the early twentieth century, osteoporosis could only be detected on plain X-rays at a late stage when bone loss was already advanced and this was associated with vertebral compression fractures (34). A major step forward towards early diagnosis of osteoporosis occurred in 1988 in the form of official approval for the use of dual-energy X-ray absorptiometry (DXA) for the diagnosis of osteoporosis, which allowed the quantification of bone mineral density (BMD) and facilitated the later development of standardized definitions for

osteoporosis and osteopenia comparing data with those of young healthy adult women (T-scores) and with data from healthy age-related controls (Z-scores).

In 1991 the since used consensus definition of osteoporosis was that of "a condition characterized by low bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture risk" (35). This descriptive definition emphasized both bone mass and bone quality, although assessment of bone quality in the clinical setting was, and remains, challenging. In 1994, a WHO Study Group introduced the since then and to date extensively used operational definition based solely on BMD, which is typically assessed at the lumbar spine and femoral neck, with results reported as T-scores and Z-scores. According to this classification, a T-score within one standard deviation (SD) of the young adult mean is considered normal, a T-score of −1 to −2.5 SD indicates osteopenia, a T-score of −2.5 SD or less indicates osteoporosis; and a T-score of −2.5 SD or less with a history of a fragility fracture(s) indicates severe osteoporosis. From that point onward, fracture risk assessment and the diagnosis of osteoporosis, and consequently also the decision to initiate treatment to prevent a first or subsequent fracture relied primarily on BMD measurements. While this BMD-based definition enabled large-scale epidemiological studies and clinical decision-making protocols, it did not account for important elements of bone quality other than BMD such as bone microarchitecture and its material properties.

Notwithstanding, a low BMD has been clearly shown to be associated with increased fracture risk, and this risk to approximately double with each SD decrease below the mean for young adults regardless of the specific site of BMD measurement or type of fracture being considered (36). BMD measurements have thus been central to clinical practice for nearly four decades, and a substantial body of evidence has established their value in the diagnosis and management of osteoporosis.

However, over the past two decades, data has shown the limitations of strictly using only BMD in the evaluation of fracture risk. Studies have thus shown that up to two-thirds of fragility fractures may occur in individuals with osteopenia or even normal BMD (36-40). DXA provides two-dimensional quantitative data regarding mineral content, bone area, and areal bone mineral density. However, bone is three-dimensional, and fracture resistance, as mentioned earlier, also depends on how bone mass is distributed, thus on its structural organization at the macro- and micro-level, as well as on its material properties and the rate and balance of bone turnover (Figures 1 and 2) (1). This limitation is particularly relevant in the presence of factors for secondary osteoporosis such as endocrine disorders in which bone quality is usually more affected than bone quantity (41), and BMD may underestimate fracture risk.

It has thus become clear that BMD measurements alone do not allow capturing the full spectrum of bone fragility as alterations in bone microarchitecture, material properties, bone turnover, and accumulated microdamage, largely unmeasurable by standard DXA, may also significantly impact on fracture risk.

Whereas DXA remains the clinical gold standard for osteoporosis diagnosis and fracture risk assessment traditionally measuring BMD at the lumbar spine and hip site, it is increasingly used as part of a broader risk assessment, as recognition of its limitations has driven the development of complementary diagnostic tools that aim to assess bone quality and strength beyond BMD, offering a more comprehensive evaluation of fracture risk (42) (Figure 2).

This includes since 2008 the development of the continuously updated **WHO fracture risk assessment tool (FRAX),** which estimates the 10-year probability of hip fracture or major osteoporotic fractures combined (hip, clinical spine, proximal humerus or forearm) for an untreated patient (aged 40-90 years). The FRAX tool integrates an increasing number of clinical risk factors potentially affecting bone strength alongside data on femoral neck BMD (43, 44), thus enhancing fracture risk assessment.

Beyond DXA and the FRAX tool, several advanced tools have been developed to provide more detailed insight into bone structure and mechanical competence (Figure 2).

Among these, the **Trabecular Bone Score (TBS**), which is a score derived from data generated by DXA scans complements BMD by providing indirect information on trabecular microarchitecture. When combined with BMD and clinical risk factors, TBS improves fracture risk prediction and is increasingly used in clinical practice to adjust Fracture Risk Assessment Tool (FRAX)-based estimates of the 10-year probability for major osteoporotic and hip fractures (43, 44, 46-48).

High-resolution peripheral quantitative computed tomography (HR-pQCT) and finite element analysis allow non-invasive three-dimensional assessment of bone at peripheral sites such as the radius and the tibia. HR-pQCT can quantify volumetric BMD and distinguish cortical from trabecular compartments, offering valuable information on bone geometry and microarchitecture. Although HR-pQCT has shown to be valuable in fracture risk prediction (3, 49, 50), its high cost, radiation exposure, and limited availability currently restrict its use largely to research settings.

Bone strength is not only dependent on BMD and bone structure to resist fracture, but also on bone material properties (1, 51-53). Historically, these material properties

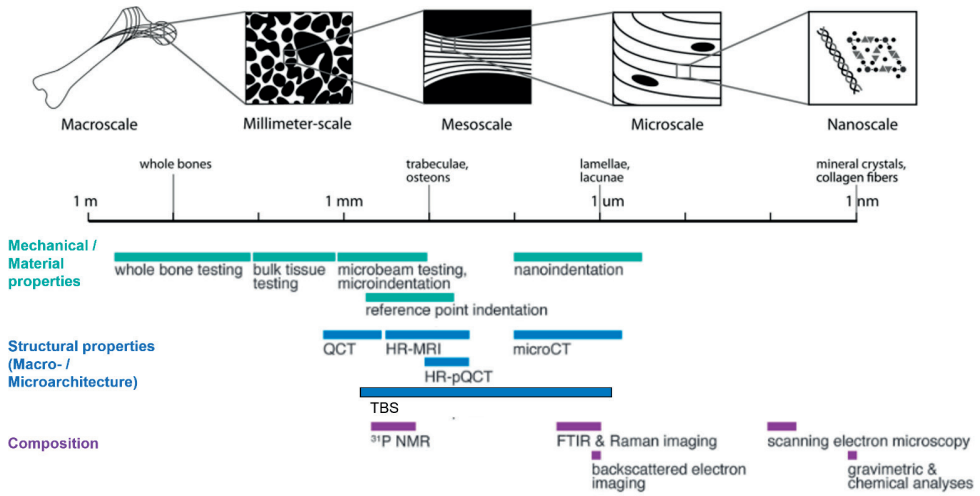


Figure 2: Hierarchical contributions to bone's resistance to fracture depicted on a logarithmic scale. Available tools and techniques for the evaluation of mechanical (green bars), structural (blue bars), and compositional (violet bars) properties of bone in the assessment of fracture risk. Mechanical testing evaluates intrinsic material properties (e.g., Reference Point Indentation for the measurement of BMSi, the parameter assessed in this thesis), imaging-based methods characterize macro- and microarchitecture (QCT: quantitative computed tomography; HR-MRI: high-resolution magnetic resonance imaging; HR-pQCT: high-resolution peripheral QCT; TBS: Trabecular Bone Score), and spectroscopic and microscopic approaches assess tissue composition (NMR: nuclear magnetic resonance imaging; FTIR: Fourier transform infrared; backscattered electron imaging, SEM). Adapted from Donnelly et al. (45).

could only be assessed ex vivo using invasive bone biopsies, usually taken from the iliac crest (52, 53). Such biopsies allow detailed histomorphometric and microarchitectural analyses, as well as assessment of bone turnover and mineralization following specialized embedding and staining procedures. In addition, advanced techniques such as quantitative backscattered electron imaging, Fourier-transform infrared spectroscopy, or nanoindentation can be applied to biopsy samples to characterize bone mineralization density distribution, collagen quality, or local tissue-level mechanical properties. Despite the wealth of information these biopsies may provide, this largely safe procedure is nevertheless invasive and this approach is limited by the discomfort patients are subjected to, the restricted anatomical site used, and its unsuitability for repeated measurements in routine clinical practice.

These limitations have motivated the development of minimally invasive, in vivo methods for assessing bone material properties. Over the past two decades, the Reference Point Indentation (RPI) technique has emerged as a valuable tool for directly evaluating tissue-level properties of bone in a minimally invasive manner in humans in vivo (54, 55). The Impact Microindentation (IMI) approach, implemented

clinically using the handheld OsteoProbe® device, is a modification of the original Reference Point Indentation technique, which relied on the larger BioDent™ system that had been mainly used for preclinical work. The primary principle of the technique is to apply a standardized force to indent the cortical bone surface, typically at a site at the mid-shaft of the tibia, in order to measure its resistance to this controlled mechanical stress. The observation that the mechanically applied stress induced microcracks similar to those found in fractured cadaveric human bone samples (55) led to FDA approval of the use of the technique for in vivo assessment of bone's ability to resist fractures in humans in 2021.

Impact Microindentation (IMI) for the in vivo assessment of bone material properties

A key method for assessing bone material properties in vivo is the Impact Microindentation (IMI) technique, performed using the handheld OsteoProbe® device (Figure 3). The procedure enables direct evaluation of cortical bone tissue properties in humans.

Measurements are typically performed at the mid-shaft of the tibia site after applying a local anesthetic, with the patient lying in the supine position (Figure 4A and B).

The probe is gently inserted into the skin and the periosteum after the bone surface is reached (Figure 5A and B). With the probe positioned perpendicular to the bone, a preload of 10 N is applied to establish contact with the surface of the bone (Figure 5C). An impact force of 30N is then delivered by the OsteoProbe® to indent the cortical bone of the tibia (Figure 5D).

The resistance of the bone tissue to this applied mechanical challenge is expressed as the Indentation Distance Increase (IDI), the distance the probe penetrates into the bone after impact. At least eight indentations are performed, with each indentation conducted 2 mm away from the previous one, without pulling the tip of the probe out of the skin for the whole duration of the procedure. Measurements are discarded when the indentation technique is poorly performed, often due to the test probe slipping on the bone surface but also due to unintentional movement of the subject's leg. After completion of the tibia measurements, five additional measurements are performed on a polymethylmethacrylate (PMMA) reference phantom (Figure 4B). The device software then calculates the outcome parameter of Impact Microindentation (IMI): the Bone Material Strength index (BMSi), which is defined as 100 times the harmonic mean of the Indentation Distance Increase (IDI) from impact into the PMMA phantom, divided by the average IDI from impact into bone tissue (Equation 1). The lower the bone strength, the deeper the probe indents the bone surface (resulting in a higher IDI), and the lower the BMSi (Figure 6).

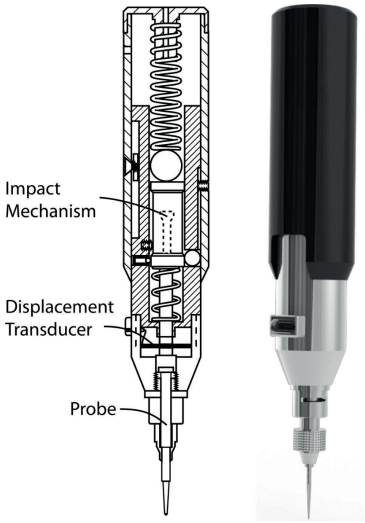
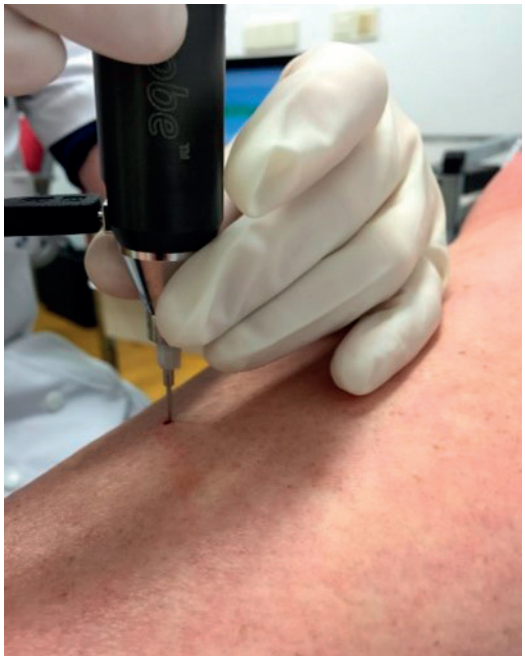


Figure 3: Schematic diagram (left) and rendering (right) of the OsteoProbe® device. The device consists of three main components: an impact mechanism, a displacement transducer, and a probe. During measurement, the impact mechanism delivers a defined force impulse that advances the probe into the bone surface. The resulting indentation depth is recorded by four strain gauges mounted on a flexible beam and connected via a bridge circuit. Adapted from Bridges et al., 2012.

A) 

B) 

Figure 4: (A) Method of Use of the OsteoProbe® on the midshaft of the tibia after the application of a local anesthetic, (B) measurement performed on the polymethylmethacrylate (PMMA) reference phantom.

$$BMSi = 100\ x\ \frac{IDI\ PMMA}{IDI\ Bone} \qquad (1)$$

IMI measurements were demonstrated to identify patients at increased fracture risk (56-58). BMSi was thus found to be lower in individuals with prior low-energy trauma fractures compared to controls (59). Despite being measured at the tibia, a site rich in cortical bone, low BMSi has been shown to be associated with increased bone fragility at all relevant skeletal sites (56, 57, 59-61), including vertebral, nonvertebral, and hip sites. Interestingly, BMSi values do not correlate with BMD values (59). There is only weak or no correlation with conventional microarchitectural parameters such as trabecular thickness, trabecular number, and cortical thickness (62, 63), highlighting its value in capturing aspects of bone material properties not accessible by standard imaging.

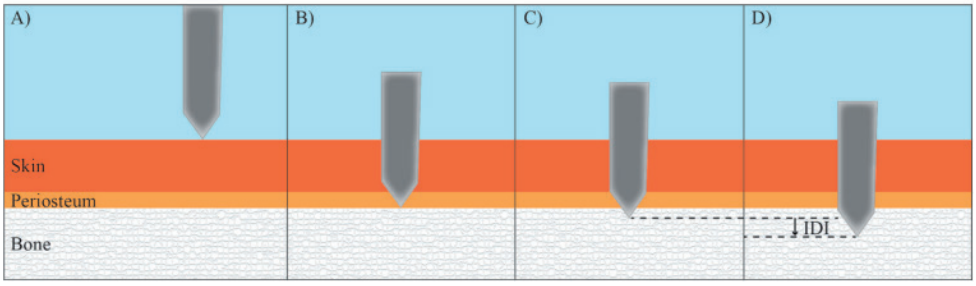


Figure 5: Illustration of the Impact Microindentation method using the OsteoProbe® device for measuring BMSi of bone.

Whereas it has been shown that data obtained by IMI reflect mechanical properties of bone that contribute to bone strength, it is as yet not fully understood which specific mechanical properties of bone are captured by IMI. Recent studies combining IMI measurements with trans-iliac bone biopsies have provided some insights into the tissue properties captured by IMI (64, 65). BMSi was thus found to negatively correlate with cortical porosity at the micro- and nano-level, and to positively correlate with organic matrix parameters such as glycosaminoglycans and pyridinoline, as well as with mineral parameters such as local mineral content and the mineral-to-matrix ratio (MM). These findings suggest that BMSi is influenced by the composition of the bone organic matrix as well as by bone mineral properties.

Experience has been accumulating with the use of IMI over the past decade, and the OsteoProbe® device is currently approved for use in the clinic in Europe, Australia and the United States (FDA approval 2021). A standard operating procedure for IMI has been published in 2016 (66), and a more recently published study proposed IMI reference intervals for men and women using data obtained from an international

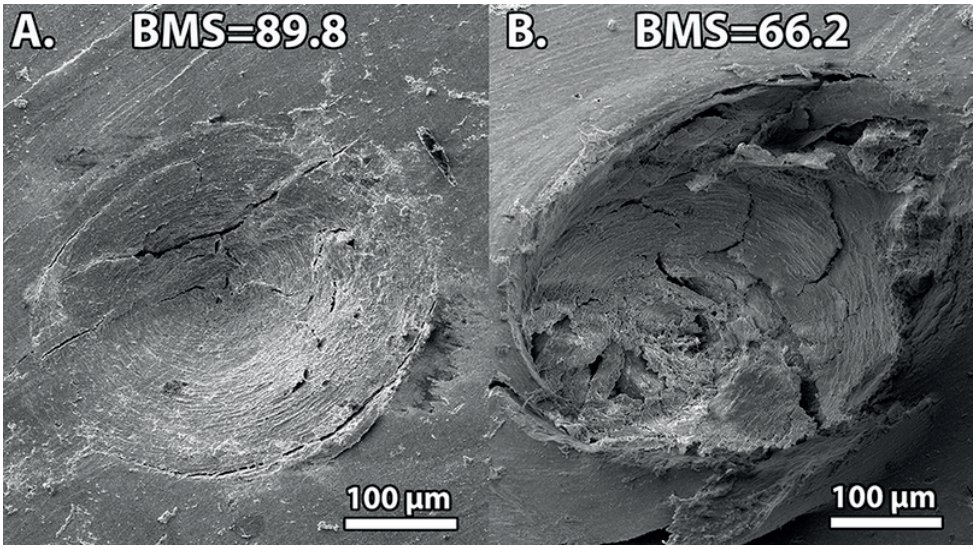


Figure 6: Scanning electron microscope (SEM) images of OsteoProbe® indentations in the tibiae of two 83-year-old female deceased donors. The images show the microcracks formed by the measurement used to determine the Bone Material Strength index (BMSi). In Sample A (left), fewer and shorter microcracks are visible on the bone surface, corresponding to a smaller indentation distance and a higher BMSi value of 89.8. In contrast, Sample B (right) shows more and longer microcracks, indicating a larger indentation distance and a lower BMSi of 66.2. These findings illustrate that bone with a higher BMSi is more resistant to local damage caused by indentation. Adapted from Randall et al., 2013

multi-center study evaluating healthy adults from several countries including subjects from our Center for Bone Quality at the Leiden University Medical Center, which is a center of excellence for the IMI technique (61).

However, despite these advances in knowledge and the accumulating data about the value of the IMI technique in the assessment of bone strength and thus of fracture risk, a number of questions still remain to be answered before the use of this tool can be recommended for routine use in clinical practice.

Outline and aims of the thesis

The increasing realization of the limitations of the sole use of DXA BMD in the evaluation of fracture risk has led to significant advances in the search for additional tools beyond DXA BMD in the assessment of bone strength. This thesis addresses the contribution of the increasingly used Impact Microindentation technique to the evaluation of bone strength and thus fracture risk in clinical practice, primarily in primary and secondary osteoporosis. To set the scene for this, we review the accu-

mulated evidence obtained from IMI performed in vivo in humans by conducting a comprehensive systematic review of all 38 clinical studies using the Impact Microindentation technique in vivo in humans up to 2019, also addressing practical aspects of the technique, which is described in the first part of this thesis in **Chapter 2**.

The second part of this thesis focusses on the evaluation of BMSi in patients with primary and secondary osteoporosis. Although several studies reported a lower BMSi in patients with primary osteoporosis and fragility fractures compared to non-fracture controls, there are no published data on bone material properties in patients with high-energy trauma fractures. In **Chapter 3,** we address this issue by conducting a cross-sectional study comparing results of IMI measurements obtained in subjects with high-energy trauma fractures with those obtained in patients with low-energy trauma fractures.

In **Chapter 4,** we conduct an observational longitudinal study to assess whether treatments of osteoporosis induce changes in BMSi as evaluated before and after treatments given for periods longer than 12 months in patients with low bone mass with and without fractures.

After exploring BMSi and its changes with treatment in patients with primary osteoporosis, we set out to study the contribution of BMSi measurements to the assessment of bone strength in patients with secondary osteoporosis associated with two endocrine diseases, in which traditional measurements of BMD usually underestimate fracture risk, primary hyperparathyroidism (PHPT), and endogenous Cushing's syndrome.

In patients with PHPT, BMD is often decreased in cortical bone but relatively preserved in trabecular bone, although fracture rate is increased at both sites, and fractures occur at higher BMD values than expected. In **Chapter 5**, we conduct a cross-sectional study to compare BMSi values among PHPT patients with and without low-energy-trauma fractures with those of non-PHPT control patients with or without low-energy trauma fractures.

In patients with Cushing's syndrome, BMD has been shown to not only underestimate fracture risk before treatment, but also to increase towards values within the normal range after treatment for hypercortisolism, although fracture risk remains increased, suggesting that BMD may not adequately reflect fracture risk in these patients. In **Chapter 6** we explore whether material properties of bone remain altered in Cushing's disease after long-term remission by conducting a cross-sectional study using impact microindentation to compare BMSi data between 60 patients with endogenous Cushing's syndrome in remission followed up at the Leiden Univer-

sity Medical Center and 60 age-, gender- and BMD-matched controls also followed up at our Center.

IMI has been increasingly used over the past decade in studies in humans to evaluate the contribution of tissue material properties to bone fragility, and the OsteoProbe® is currently approved for use in the clinic in Europe, the United States and Australia. Previous data show that IMI is well tolerated during the procedure and not associated with side effects immediately following it. However, the procedure is minimally invasive and there are no data available on the longer-term safety and acceptability of this technique. Therefore, in the last study included in the third part of this thesis, described in **Chapter 7**, we address these issues by prospectively inviting patients with primary or secondary osteoporosis who took part in the studies included in this thesis to report any minor or major event related to the procedure. This was conducted in person immediately after the procedure and followed by a telephone interview 7 and 30 days thereafter. The primary outcome was the complication rate 30-day after the IMI procedure.

In the last part of this thesis, a Summary of the results of all the studies is provided in **Chapter 8**, followed by a General Discussion and Future perspectives with the use of the technique in **Chapter 9**. A Dutch summary of the thesis is provided in **Chapter 10**.

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