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

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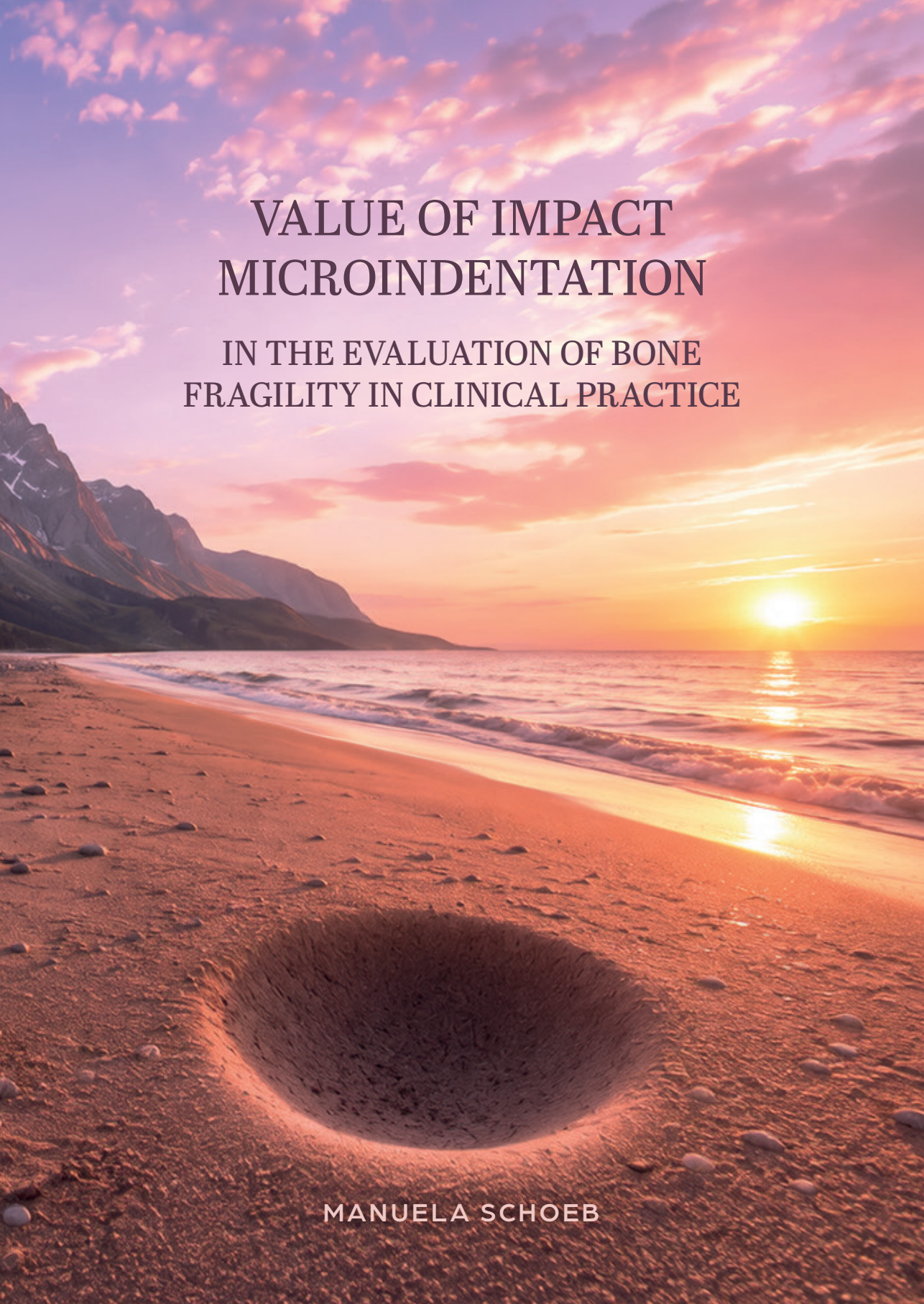

VALUE OF IMPACT MICROINDENTATION

IN THE EVALUATION OF BONE FRAGILITY IN CLINICAL PRACTICE

MANUELA SCHOEB

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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.

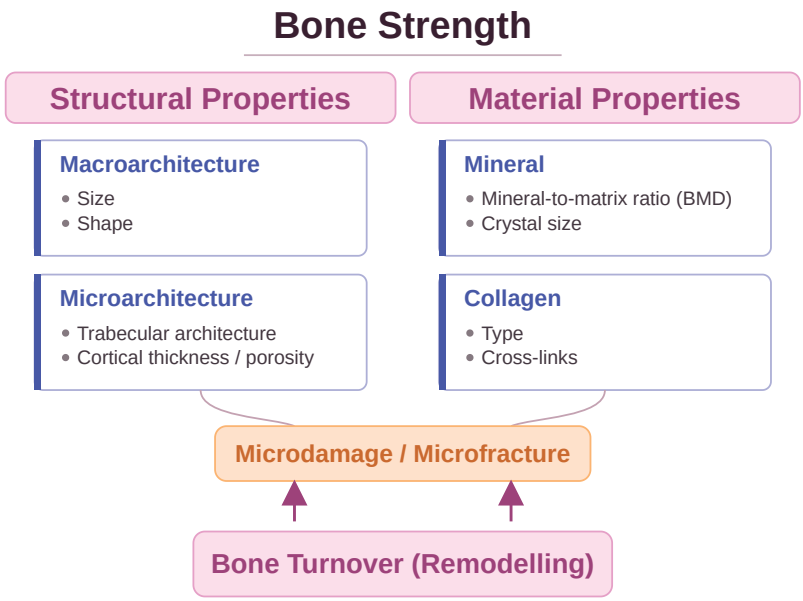


Figure 1. Determinants of bone strength. Bone strength is determined by the interaction between structural properties, material properties of the bone matrix, and bone turnover (remodelling). Disruption of any of these components may compromise the ability of bone to resist fracture.

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

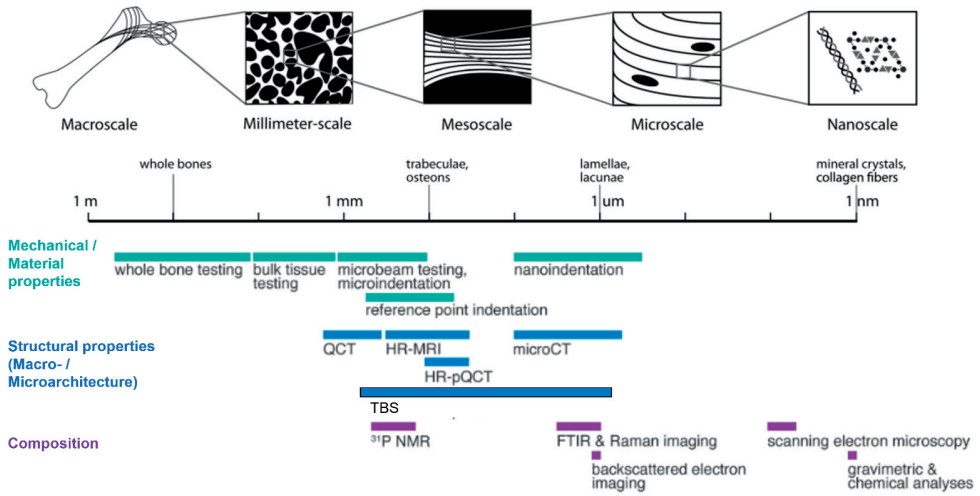


Figure 2: Hierarchical contributions to bone's resistance to fracture depicted on a logarithmic scale. Available tools and techniques for the evaluation of mechanical (green bars), structural (blue bars), and compositional (violet bars) properties of bone in the assessment of fracture risk. Mechanical 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).

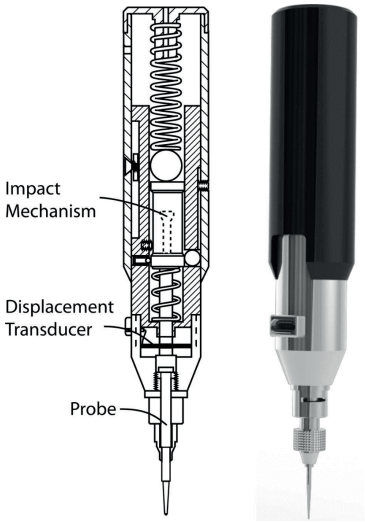
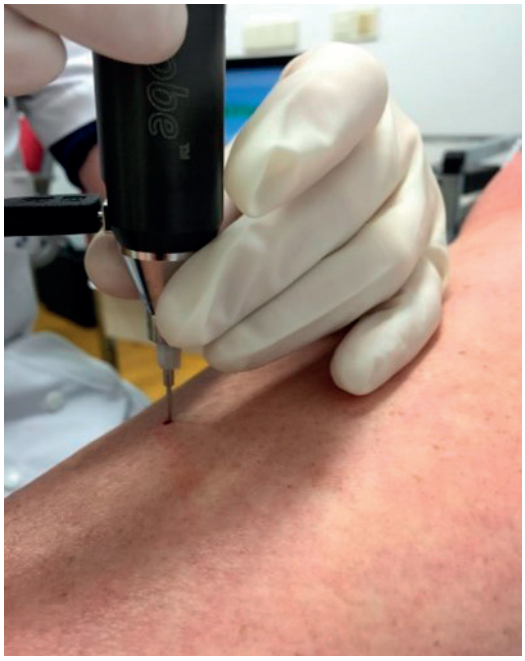


Figure 3: Schematic diagram (left) and rendering (right) of the OsteoProbe® device. The device consists of three main components: an impact mechanism, a displacement transducer, and a probe. During measurement, the impact mechanism delivers a defined force impulse that advances the probe into the bone 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.

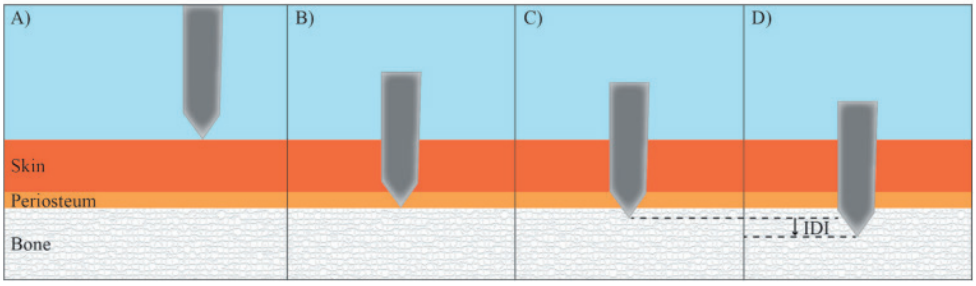


Figure 5: Illustration of the Impact Microindentation method using the OsteoProbe® device for measuring BMSi of bone.

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

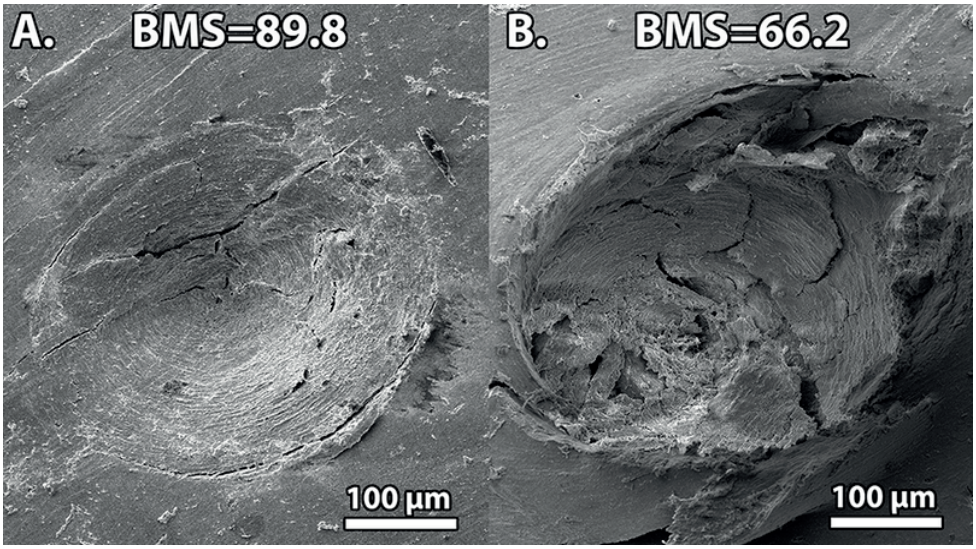


Figure 6: Scanning electron microscope (SEM) images of OsteoProbe® indentations in the tibiae of two 83-year-old female deceased donors. The images show the microcracks formed by the measurement used to determine the Bone Material Strength index (BMSi). In Sample A (left), fewer and shorter microcracks 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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Chapter 2

Added Value of Impact Microindentation in the Evaluation of Bone Fragility: A Systematic Review of the Literature

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Abstract

The current gold standard for the diagnosis of osteoporosis and the prediction of fracture risk is the measurement of bone mineral density (BMD) using dual energy x-ray absorptiometry (DXA). A low BMD is clearly associated with increased fracture risk, but BMD is not the only determinant of bone strength, particularly in secondary osteoporosis and metabolic bone disorders in which components other than BMD are affected and DXA often underestimates true fracture risk. Material properties of bone which significantly contribute to bone strength have become evaluable *in vivo* with the impact microindentation (IMI) technique using the OsteoProbe® device. The question arises whether this new tool is of added value in the evaluation of bone fragility. To this effect, we conducted a systematic review of all clinical studies using IMI *in vivo* in humans also addressing practical aspects of the technique and differences in study design, which may impact outcome. Search data generated 38 studies showing that IMI can identify patients with primary osteoporosis and fractures, patients with secondary osteoporosis due to various underlying systemic disorders, and scarce longitudinal data also show that this tool can detect changes in bone material strength index (BMSi), following bone-modifying therapy including use of corticosteroids. However, this main outcome parameter was not always concordant between studies. This systematic review also identified a number of factors that impact on BMSi outcome. These include subject- and disease-related factors such as the relationship between BMSi and age, geographical region and the presence of fractures, and technique- and operator-related factors. Taken together, findings from this systematic review confirm the added value of IMI for the evaluation and follow-up of elements of bone fragility, particularly in secondary osteoporosis. Notwithstanding, the high variability of BMSi outcome between studies calls for age-dependent reference values, and for the harmonization of study protocols. Prospective multicenter trials using standard operating procedures are required to establish the value of IMI in the prediction of future fracture risk, before this technique is introduced in routine clinical practice.

INTRODUCTION

Bone fragility is complex and its evaluation represents a significant challenge in clinical practice. The tools used to assess bone strength and thus fracture risk have so far included the measurement of bone mineral density using dual energy x-ray absorptiometry (DXA) and the evaluation of clinical risk factors for increased bone fragility using the FRAX algorithm. BMD measurements have been routinely performed in the clinic for over three decades and experience with their use is substantial. A low BMD has been clearly associated with increased fracture risk, but evidence has been accumulating over the past decade for factors contributing to bone strength other than BMD, as only one third of an individual's fracture risk is being explained by BMD values (1). The strength of bone thus not only depends on bone mineral density but also on its architecture at the macro-, micro- and nanolevel and on its material composition (2). Available tools for the evaluation of these various components of bone strength have so far included primarily histological evaluation of bone biopsies to assess bone histomorphometry parameters and nanoindentation to assess material properties of bone. Other tools more recently included high resolution peripheral quantitative computed tomography (HR-pQCT) to assess bone structure, and finite element analysis (3, 4). However, these methods are either invasive and time-consuming in their analysis, or associated with high radiation exposure. Material properties of bone, which significantly contribute to bone strength could until recently only be assessed *ex vivo* on a transiliac bone biopsy specimen. Since the introduction of Reference Point Indentation, the possibility has emerged for directly evaluating tissue-level properties of bone in humans *in vivo* (5, 6).

Impact microindentation (IMI) using the handheld OsteoProbe® device has been developed for use in the clinic as an adaption of the original Reference Point Indentation technique (7). Experience has been accumulating with the use of this technique and data have been collected from an increasing number of patients mainly with primary or secondary osteoporosis. A standard operating procedure for IMI has also been recently published to harmonize collection of data (8). Although results have been so far promising, outcomes have not always been concordant between studies or centers so that the added value of this technique in the evaluation of bone fragility still remains to be established. To address this issue, we conducted a systematic review of the literature of all clinical studies in which impact microindentation was performed *in vivo* in humans using the OsteoProbe® device including those published as meeting abstracts. Our objective was to assess the potential added value of impact microindentation in the evaluation of fracture risk in clinical practice. In this process, we also reviewed available literature on practical aspects of the technique and on differences in study design, which may explain differences in outcome between studies, adding new data to those studies published in the last review on the topic in 2017 (9).

The Reference Point Indentation Technique

Reference Point Indentation (RPI) is a technique which enables the assessment of material properties of bone by indenting the bone surface of the tibia *in vivo* in humans. The principle of RPI is based on the hypothesis that indentation of the bone surface results in separation of mineralized collagen microfibers, resulting in microcracks (10). The observation that RPI induced microcracks similar to those observed in fractured cadaveric human bone samples led to the development of the technique *in vivo* in humans in 2006 with a view to assessing the ability of bone to resist fractures. Two Reference Point Indentation devices have so far been used. The original Biodent™ device has been used in the laboratory in animal studies, on human cadaveric bone, and also in early studies *in vivo* in humans (10, 11). The handheld device OsteoProbe® was adapted from the Biodent™ device for *in vivo* use in large animals and in the clinic (7). To avoid confusion in the interpretation of data, a different nomenclature has been proposed for the two devices in 2016 using the term cyclic reference point microindentation (CMI) for the Biodent™ device, and impact microindentation (IMI) for the OsteoProbe® device (8). Since the OsteoProbe® is currently the only tool used in the clinic, and there have been no new publications using the Biodent™ *in vivo* in humans since 2013, with all prior publications included in the last review (9), this systematic review of the impact microindentation technique covers published literature of studies only using the OsteoProbe®.

Using the OsteoProbe®, measurements are performed at a localization at the mid-shaft of the tibia after applying local anesthetic with the patient in the supine position (**Figure 1A**). The probe is gently inserted in the skin at the point of interest until the bone surface is reached, following which the cortical bone of the tibia is indented by an impact delivered by the OsteoProbe®. The resistance of bone tissue to this applied mechanical challenge is expressed as the measured distance covered by the test probe after impact of the probe into bone: the Indentation Distance Increase (IDI). Poorly performed measurements, usually due either to slipping of the test probe on the surface of the bone or to unintentional movement of the subject's leg, are discarded.

After 10 adequate measurements are obtained, five further measurements are performed on a polymethylmethacrylate (PMMA) reference phantom (**Figure 1B**). The software then calculates the outcome parameter of IMI: the Bone Material Strength index (BMSi). BMSi is defined as 100 times the harmonic mean of IDI from impact into the PMMA phantom divided by the average IDI from impact into bone tissue (7). The lower bone strength is, the deeper the probe indents the bone surface (high IDI) and the lower is the outcome parameter BMSi.

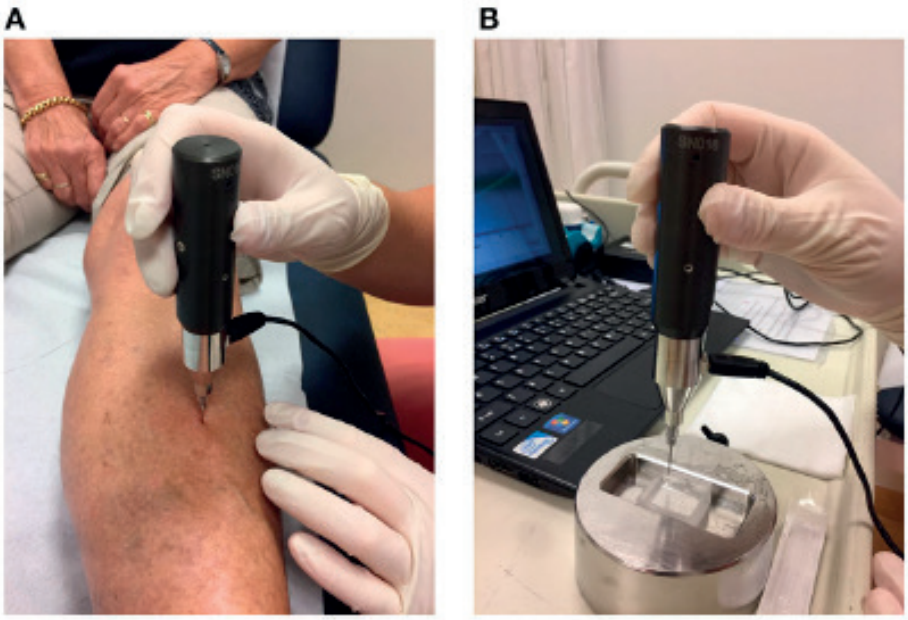

FIGURE 1 | (A) Method of use of the OsteoProbe® on the midshaft of the tibia after the application of a local anesthetic, (B) measurement performed on the polymethylmethacrylate (PMMA) reference phantom.

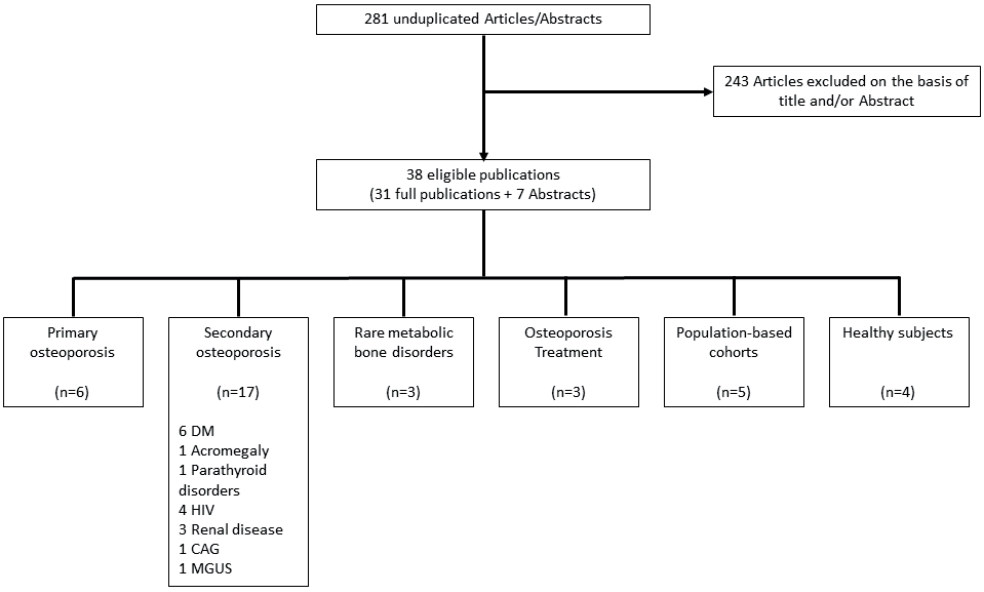

FIGURE 2 | Flowchart of study selection. DM, Diabetes mellitus; HIV, Human immunodeficiency virus; CAG, Chronic atrophic gastritis; MGUS, Monoclonal gammopathy of undetermined significance.

Search Strategy and Eligibility Criteria

A search strategy was designed with the help of an experienced librarian for the systematic review of the literature on impact microindentation. The search, which was conducted in PubMed, EMBASE and Web of Science, included all original published articles and meeting abstracts in the English language, updated up to August 2019. Relevant keywords were used, including free text words (see Supplementary Material for details of complete search). Studies using the cyclic RPI technique with the pre-clinical device Biodent™, and studies using early OsteoProbe I™ and II™ devices were excluded from analyses. All articles identified by the search were assessed by two independent investigators.

Search Data Extraction

Studies were independently reviewed by the two independent investigators and the following data were extracted: (1) Demographic data on patient and control populations (clinical characteristics, population size); (2) outcome data (BMSi and BMD at the femoral neck (FN)); (3) Factors potentially influencing BMSi outcome (age, gender, body mass index (BMI), geographical region, prevalent fractures, BMD, number of operators, intra- and interobserver coefficient of variation, number of indentations per BMSi obtained); (4) potential complications arising from IMI. Extracted data were reported as presented in the articles, as mean ± SD, mean (SEM), or median (IQR).

Quality Assessment

The quality of full publications was independently assessed using the Newcastle-Ottawa (NO) Scale, which includes three domains: selection, comparability and exposure/outcome and was adapted for this review (see Supplementary Table). Based on the total quality score, articles were scored out of a maximum score of ten as unsatisfactory (0–4), satisfactory (5–7), or good (8–10).

RESULTS

Study Selection

The search yielded 456 articles and 102 meeting abstracts. Two hundred and seventy-seven duplicate articles in ≥2 electronic data bases were excluded, and 281 unique studies were further assessed; (**Figure 2**). Two hundred and forty-three of these were excluded on the basis of title and abstract: 218 studies were not using the OsteoProbe®, eight were using the OsteoProbe® either in animal studies (12–14) or in *ex vivo* human bone (15–19), two described primarily technical aspects of RPI devices (8, 20) and 15 were review articles, eight describing different techniques for the assessment of bone quality including RPI (21–28), and seven specifically on RPI

(7, 9, 20, 29–32). Included in the final evaluation were 31 original articles and seven studies published only as meeting abstracts using the OsteoProbe® microindentation device *in vivo* in humans.

Quality Assessment

Based on the adapted Newcastle-Ottawa Scale (adapted NO Scale: Supplementary Table), methodological quality of studies was good in eight studies (33–40), satisfactory in 15 studies (41–55), and unsatisfactory in six studies (56–61) (**Tables 1**–**6**). The NO Scale could not be used for the evaluation of two fully published studies (67, 69) and for all meeting abstracts (62–66, 68, 70) because of incomplete data. Of the 31 full articles, 8 (25.8%) were thus of unsatisfactory methodological quality or unevaluable. Notwithstanding, for the purpose of completeness of this systematic review of the literature we report on all full publications as well as meeting abstracts yielded by our search strategy reporting the use of IMI *in vivo* in humans.

Characteristics of Studies Generated by the Search

Of the 38 reported studies using the OsteoProbe®, 17 included patient groups with a known increased risk for fragility fractures on the basis of secondary osteoporosis, although this was not fully reflected by BMD values (36, 41, 45–49, 52, 53, 56, 58, 59, 62–66). The majority of these studies included patients with endocrine disorders such as diabetes mellitus and acromegaly (n = 8) (36, 41, 48, 49, 62–65). Six studies focused on patients having sustained fragility or stress fractures (33–35, 38, 50, 55), three studies evaluated BMSi outcomes in patients receiving osteoporosis treatment (37, 44, 68) and another three studies reported on patients with a rare metabolic bone disorder: Type 1 Gaucher Disease, Paget's and Camurati-Engelmann disease (57, 60, 67). Seven studies, two of which included patients with diabetes mellitus (41, 63), were performed in two population-based cohorts from Sweden and Australia, both designed to assess epidemiological data in osteoporosis (39, 43, 51, 54, 69). Four other studies included healthy individuals (40, 42, 61, 70) (**Figure 2**).

The number of patients in whom BMSi was measured varied greatly between studies, with the smallest study including only seven subjects (68) and the largest 489 subjects (41). All studies were performed in adults with ages ranging from a median of 33.9 years (27.6–53.8 years) (46) to a mean of 78.3 ± 1.1 years (51). Fourteen studies included only women (35, 37–43, 48–51, 55, 68), and four studies included only men (54, 63, 64, 69). Although not always explicitly stated, 17 studies appear to have overlapping patient or control cohorts (33, 36, 39, 41, 43, 45, 51–54, 57–60, 63, 67, 69).

TABLE 1 | Impact microindentation studies in subjects with osteoporosis/fractures.

References	Subjects	(m/f)	Age (years)	FN BMD					
(g/cm2)	BMSi	Relationship of BMSi with			Study quality scale (0–10)				
						Age	BMD	Fx	
Malgo et al. (33)	Fx	63 (24/39)	62.6 ± 9.6 (40–85)	0.67 ± 0.09	79.9 (0.6) (78.7–81.1)	Neg	No	Pos	8
	No Fx	27 (13/14)	57.1 ± 9.5 (40–85)	0.69 ± 0.08	82.4 (1.0) (80.3–84.5)				
Malgo et al. (34)	NVF only	53 (14/39)	62.8 ± 8.3 (40–85)	0.65 ± 0.07	78.9 (0.7) (77.5–80.3)	Neg	No	Pos	9
	VF + NVF	34 (14/20)	62.8 ± 9.9 (40–85)	0.69 ± 0.09	78.3 (0.9) (76.5–80.1)				
	VF only	14 (8/6)	64.7 ± 9.3 (40–85)	0.70 ± 0.09	78.4 (1.4) (75.4–81.4)				
	No Fx	31 (11/20)	57.5 ± 9.9 (40–85)	0.68 ± 0.07	82.5 (0.9) (80.7–84.3)				
Duarte Sosa and Fink Eriksen (38)	Stress Fx	30 (0/30)	39.0 ± 13.9 (19–64)	0.92 ± 0.25	70.5 ± 8.7 (67.4–73.6)	No	No	Pos	8
	No Fx	30 (0/30)	42.3 ± 9.8 (23–66)	1.05 ± 0.11	77.1 ± 7.2 (74.5–79.7)				
Sosa and Eriksen (35)	NH/NVF	17 (0/17)	66.2 ± 9.1 (50–85)	0.77 ± 0.07	73.1 ± 6.5 (69.8–76.4)	No	No	Pos	10
	HF	25 (0/25)	68.2 ± 10.0 (50–85)	0.76 ± 0.11	72.0 ± 6.5 (69.3–74.7)				
	VF	24 (0/24)	67.8 ± 10.1 (50–85)	0.71 ± 0.10	70.1 ± 7.1 (67.2–73.0)				
	No Fx	66 (0/66)	66.5 ± 7.9 (50–85)	1.02 ± 0.13	76.4 ± 6.2 (74.9–77.9)				
Rozental et al. (50)	DRF	57 (0/57)	64.2 ± 10.5 (>50)	0.68 ± 0.11	74.4 ± 8.8 (72.1–76.7)	Neg	Pos	DRF Pos	6
	HF	42 (0/42)	75.7 ± 10.9 (>50)	0.61 ± 0.12	74.6 ± 8.5 (72.0–77.2)			HF No	
	No Fx	93 (0/93)	67.3 ± 7.6 (>50)	0.72 ± 0.10	77.4 ± 8.8 (75.6–79.2)				
Popp et al. (55)	AFF	15 (0/15)	71.8 ± 10.8	0.69 ± 0.03	76.5 ± 10.9 (70.5–82.5)	No	No	No	5
	HF	20 (0/20)	74.6 ± 8.3	0.62 ± 0.03	78.3 ± 9.3 (73.9–82.7)				
	BP >5 years	30 (0/30)	71.9 ± 9.1	0.60 ± 0.19	76.6 ± 10.5 (72.8–80.4)				
	BP–naive	88 (0/88)	65.9 ± 5.6	0.71 ± 0.01	80.1 ± 8.3 (78.4–81.8)				

Age is presented as mean ± SD (range, if reported), BMD is presented as mean ± SD and BMSi is presented as mean ± SD (95% CI) or mean (SE) (95% CI). 95% CI calculated from data provided in the publication. AFF, Atypical femoral fracture; BMD, Bone mineral density; BMSi, Bone material strength index; BP, Bisphosphonate; DRF, Distal radius fracture; FN, Femoral neck; Fx, Fracture; HF, Hip fracture; NH/NVF, Non-hip non-vertebral fracture; NVF, Non-vertebral fracture; VF, Vertebral fracture.

TABLE 2 | Impact microindentation studies in subjects with secondary osteoporosis.

(a) Endocrine disorders										
References	**Subjects**	**(m/f)**	**Age (years)**	**FN BMD (g/cm²)**	**Fx prevalence (%)**	**BMSi**	**Relationship of BMSi with**			**Study quality scale (0–10)**
							Age	**BMD**	**Disease**	
Farr et al. (48)	Type 2 DM	30 (0/30)	65.4 (1.4)	0.93 (0.03)	10.0	77.2 (1.6) (73.9–80.6)	NA	NA	Lower in DM	5
	Controls	30 (0/30)	65.7 (1.6)	0.94 (0.03)	10.0	85.7 (1.6) (82.4–89.0)				
Nilsson et al. (41)	Type 2 DM	99 (0/99)	77.6 ± 1.5 (75–80)	0.69 ± 0.10	55.0	74.6 ± 7.6* (72.5–76.7)	NA	NA	Lower in DM	7
	Controls	954 (0/954)	77.7 ± 1.5 (75–80)	0.66 ± 0.10*	52.0	78.2 ± 7.5* (75.5–78.9)				
Furst et al. (49)	Type 2 DM	16 (0/16)	65.4 ± 2.4	0.77 ± 0.03	19.0	63.7 (1.9) (59.7–67.8)	NA	No	Lower in DM	7
	Controls	19 (0/19)	65.6 ± 1.2	0.69 ± 0.01	11.0	70.1 (1.9) (66.1–74.1)				
Barnouin et al. (62) (Ab)	Type 2 DM	27 (NA)	NA (65–85)	NA	NA	70.5 ± 6.5 (67.9–73.1)	NA	NA	NA	NA
Holloway et al. (63) (Ab)	DM	34 (34/0)	NA (33–92)	0.97 (0.92–1.01)	NA	80.6 (78.9–82.9)	NA	NA	Lower in DM, but not in IFG	NA
	IFG	37 (37/0)		0.95 (0.91–0.99)		83.6 (81.7–85.6)				
	Controls	140 (140/0)		0.96 (0.94–0.98)		83.4 (82.4–84.4)				
Syversen et al. (64) (Ab)	Type 1 DM	33 (33/0)	42.7 ± 12.1	NA	NA	NA	NA	NA	Lower in DM	NA
	Controls	28 (28/0)	41.8 ± 12.0							
Malgo et al. (36)	Acromegaly	48 (26/22)	60.2 ± 11.0	0.84 ± 0.16	58.0	79.4 (0.7) (78.0–80.8)	Pos	No	Lower in Acromegaly	8
	Controls	44 (22/22)	60.5 ± 8.5	0.80 ± 0.09	16.0	83.2 (0.7) (81.8–84.6)	Neg	No		
Starr et al. (65) (Ab)	PHPT	13 (4/9)	59.3 ± 15.0	NA	NA	67.8 ± 9.0 (62.3–73.2)	NA	NA	Lower in PHPT + HypoPT	NA
	HypoPT	15 (4/11)	44.3 ± 12.5			68.4 ± 10.0 (62.9–73.9)				
	Controls	22 (5/17)	49.2 ± 17.0			77.2 ± 8.0 (73.7–80.7)				

(b) HIV, Chronic kidney disease, CAG, MGUS, CROSS-SECTIONAL DESIGN										
References	**Subjects**	**(m/f)**	**Age (years)**	**FN BMD (g/cm²)**	**Fx prevalence (%)**	**BMSi**	**Relationship of BMSi with**			**Study quality scale (0–10)**
							Age	**BMD**	**Disease**	
Guerri-Fernandez et al. (46)	HIV	50 (35/15)	36.7 [31.7–46.2]	0.81 [0.77–0.88]	4.0	84.5 [83.0–87.0]	NA	NA	Lower in HIV	7
	Controls	35 (24/11)	33.9 [27.6–53.8]	0.79 [0.73–0.96]	0	90.0 [88.5–93.0]				
Guerri-Fernandez et al. (47)	HIV >5 years TDF/FTC	36 (27/9)	56.4 ± 6.3	0.72 [0.2]	5.5	81.0 [0.8]	No	No	Lower in TDF/FTC	7
	HIV >5 years ABC/3TC	27 (20/7)	63.0 ± 9.8	0.74 [0.2]	3.7	82.7 [1.3]				
Perez-Saez et al. (45)	ESRD before KT	53 (25/28)	55.8 ± 12.1	0.73 ± 0.15	26.4	79* [71.8–84.2]	NA	NA	Lower in ESRD	5
	Controls	94 (20/74)	50.2 ± 16.0	0.78 ± 0.12	0	82.6 [77.5–88.9]				
Perez-Saez et al. (58)	KT recipients >10 years after KT	40 (17/23)	63.8 ± 11.1	0.67 ± 0.13*	32.5	79.1 ± 7.7* (76.7–81.5)	NA	No	No	4
	Controls	94 (20/74)	50.2 ± 16.0	0.78 ± 0.12*	0	82.9 ± 7.8* (81.3–84.5)				
Aasarod et al. (56)	CAG	17 (9/8)	54.1 ± 12.6 (20–70)	0.79 ± 0.14	41.1	82.0 ± 9.6* (76.5–87.5)	NA	NA	No	1
	Controls	41 (20/21)	53.2 ± 11.4 (20–70)	0.80 ± 0.16	44.0	80.0 ± 7.0* (76.5–83.5)				
Gonzalez et al. (66) (Ab)	MGUS	22 (NA)	NA	0.73	NA	68.3 ± 5.0 (66.1–70.5)	NA	NA	Lower in MGUS	NA
	Controls	NA		0.78		83.0 ± 4.0				

TABLE 2 | Continued

References	Intervention	Subjects	(m/f)	Age (years)	FN BMD (g/cm²)	Fx prevalence (%)	BMSi	Relationship of BMSi with			Study quality scale (0–10)
								Age	BMD	Intervention	
LONGITUDINAL DESIGN											
Guerri-Fernandez et al.	ART with TDF/FTC, FU 24 (not shown) + 48 weeks	HIV	40 (33/7)	38 ± 9	BL: 0.84 ± 0.12 FU: 0.81 ± 0.11	5.0	BL: 86.1 ± 6.1 (84.2–88.0) FU: 89.0 ± 4.2 (87.7–90.3)	Neg	Pos	Increase with ART	7
Lerma-Chippirraz et al.	ART with TDF/FTC, FU 48 weeks (HIV only)	HIV	20 (16/4)	37 [31–43]	BL: 0.84 [0.79–1.02] FU: 0.82 [0.73–0.96]	0	BL: 86 [83–90] FU: 90 [88–93]	NA	NA	Increase with ART	4
		Controls	20 (15/5)	38 [35–42]	BL: 0.83 [0.75–0.98]	0	BL: 89 [88–93]				
Perez-Saez et al. (53)	Low-dose GC after KT, FU 3 (not shown) + 12 months	ESRD receiving KT	36 (19/17)	54.9 ± 11.6	BL: 0.75 ± 0.15 FU: 0.73 ± 0.14	16.7	BL: 79.2* [73.2–85.4] FU: 80.1 [73.0–85.4]	NA	NA	No	6

Age is presented as mean ± SD (range, if reported), mean (SE) or median [IQR], BMD is presented as mean ± SD, mean (95% CI), median [IQR] or median (SE) and BMSi is presented as mean ± SD (95% CI), mean (SE) (95% CI) or median [IQR]. 95% CI calculated from data provided in the publication. Ab, published only in Abstract form; ABC/3TC, Abacavir/lamivudine; ART, Antiretroviral therapy; BL, Baseline; BMD, Bone mineral density; BMSi, Bone material strength index; CAG, Chronic atrophic gastritis; ESRD, End-stage renal disease; FN, Femoral neck; FU, Follow-up; Fx, Fracture; IFG, Impaired fasting glucose; HypoPT, Hypoparathyroidism; MGUS, Monoclonal gammopathy of undetermined significance; PHPT, Primary hyperparathyroidism; DM, Diabetes mellitus; TDF/FTC, Tenofovir/emtricitabine. *Measured in a subgroup of subjects.

TABLE 3 | Impact microindentation studies in subjects with rare metabolic bone disorders.

References	Subjects	(m/f)	Age (years)	FN BMD (g/cm²)	Fx prevalence (%)	BMSi	Relationship of BMSi with			Study quality scale (0–10)
							Age	BMD	Disease	
Herrera et al. (57)	Gaucher's disease	16 (7/9)	51.3 ± 14.4 (21–89)	NA	0.0	72.7 ± 10.0 (67.4–78.0)	NA	NA	Lower in Gaucher's disease	4
	Controls	29 (5/24)	48.7 ± 15.8 (20–69)		0.0	81.8 ± 1.4 (81.3–82.3)				
Malgo et al. (67)	Unilateral Paget's disease of the tibia	9 (4/5)	69.5 (55–87)	NA	NA	Pagetic Tibia: 74.7 (1.7) (70.8–78.6) Non-pagetic Tibia: 78.7 (1.3) (75.7–81.7)	NA	NA	NA	NA
	Controls	11 (7/4)	61.9 (51–72)	NA	72.7	Dominant Tibia: 82.1 (1.3) (79.2–85.0) Non-dominant Tibia: 81.4 (1.3) (78.5–84.3)				
Herrera et al. (60)	Camurati-Engelmann	3 (1/2)	44.0 [43–47]	NA	0.0	76.9	NA	NA	Lower in Camurati-Engelmann (ns)	2
	Controls	29 (5/24)	56.0 (NA)		0.0	81.4				

Age is presented as mean ± SD (range), mean (range) or median [range] and BMSi is presented as mean ± SD (95% CI), mean (SE) (95% CI) or median. 95% CI calculated from data provided in the publication. BMD, Bone mineral density; BMSi, Bone material strength index; FN, Femoral neck; Fx, fracture.

Impact Microindentation Studies in Patients with Fractures (Table 1)

Initial studies using IMI aimed at establishing the value of the novel technique in the evaluation of bone fragility in patients who had sustained a fracture or were at high risk of sustaining one. Six of these studies evaluated the association between BMSi and prevalent fractures (33–35, 38, 50, 55).

Confirming data initially obtained by the Spanish group using the earlier device Biodent™ (10, 11), our group demonstrated that in the presence of comparable BMD values, BMSi was lower in 63 patients who had sustained a fragility fracture compared to 27 patients who had not [respectively, 79.9 (SE 0.6) vs. 82.4 (SE 1.0), $p = 0.032$]. BMSi was also comparable in patients with fragility fractures irrespective of whether they had osteopenia or osteoporosis (33). A subsequent study conducted in 132 patients, including data from the 90 patients from the original publication, confirmed that BMSi was lower in patients with fragility fractures ($n = 101$) compared to those who never sustained a fracture ($n = 31$) [respectively, 79.0 (SE 0.5) vs. 82.5 (SE 0.9), $p = 0.001$], independently of BMD measurements. Interestingly we also observed that measured BMSi values were comparable in fracture patients, regardless of type of fracture: non-vertebral ($n = 53$) [BMSi 78.9 (SE 0.7)], vertebral ($n = 14$) [BMSi 78.4 (SE 1.4)] or combined non-vertebral and vertebral fractures ($n = 34$) [BMSi 78.3 (SE 0.9)] (34).

Duarte Sosa et al. confirmed these findings by demonstrating that 66 postmenopausal women with osteoporosis and fragility fractures had significantly lower BMSi than age-matched postmenopausal women who had normal BMD values and no fractures (respectively, 71.5 ± 7.3 vs. 76.4 ± 6.2, $p = 0.008$) (35). Also in keeping with results from our group (34) there was no difference in BMSi whether patients had vertebral fractures ($n = 24$), hip fractures ($n = 25$) or non-hip non-vertebral fractures ($n = 17$), with BMSi values of 70.1 ± 7.1 vs. 72.0 ± 6.5 vs. 73.1 ± 6.5, respectively (35). Findings from this study also demonstrated that BMSi was inversely related to severity of vertebral fractures as evaluated by Genant's grading score in the 24 patients with vertebral fractures ($r^2 = 0.19$, $p < 0.05$), although these results could not be reproduced by our group or by that of Rudäng et al. (34, 43). Another study from the Norwegian group excluding patients with osteoporosis or previous low-energy fractures, but including women with stress fractures of the lower extremities or pelvis, demonstrated significantly lower BMSi in 30 patients with stress fractures (BMSi 70.5 ± 8.7), than in controls without fractures (BMSi 77.1 ± 7.2, $p = 0.01$) (38).

In a cohort of 192 postmenopausal women, Rozental et al. showed that BMSi was lower in addition to lower BMD at the FN and LS in 57 patients with distal radius fractures than in 93 fracture-free controls (BMSi, respectively, 74.4 ± 8.8 vs. 77.4

± 8.8, p = 0.04) (50). This was also the case for the 42 patients with hip fractures although this did not reach statistical significance compared to controls (BMSi 74.6 ± 8.5, p = 0.09).

In a study addressing whether cortical tissue properties of bone are altered in atypical femoral fractures (AFF), BMSi was measured in 15 postmenopausal women with this rare fracture. In these patients, BMSi was lower, albeit not significantly, compared to that in 20 patients with hip fractures, 30 longterm bisphosphonate users, and 88 patients never treated with antiresorptives (BMSi 76.5 ± 10.9 vs. 78.3 ± 9.3 vs. 76.6 ± 10.5 vs. 80.1 ± 8.3, respectively) (55).

In summary, BMSi was found to be lower in all patients with fragility fractures compared to non-fracture controls independently of site of fracture and generally independently of BMD values, suggesting that tissue material properties of bone are altered in fragility fracture patients and that BMSi measured at the tibia is associated with increased bone fragility at all relevant skeletal sites.

TABLE 4 | Impact microindentation studies in subjects receiving osteoporosis treatment.

CROSS-SECTIONAL DESIGN

References	Subjects	(m/f)	Age (years)	FN BMD (g/cm²)	BMSi	Relationship of BMSi with			Study quality scale (0–10)
						Age	BMD	Fx	
Nogues et al. (37)	BP >4 years Fx	21 (0/21)	69.5 ± 5.9	0.62 ± 0.08	73.8 ± 6.5 (70.8–76.8)	NA	No	Pos	8
	BP >4 years No Fx	19 (0/18)	71.5 ± 6.8	0.66 ± 0.09	81.6 ± 6.3 (78.5–84.7)				

LONGITUDINAL DESIGN

References	Intervention	OP treatment	(m/f)	Age (years)	FN BMD (g/cm²)	Fx prevalence (%)	BMSi	Relationship of BMSi with			Study quality scale (0–10)
								Age	BMD	Intervention	
Mellibovsky et al. (44)	GC and OP prophylaxis, FU 7 and 20 weeks (not shown)	Ca/Vit D	19 (11/8)	55.3 ± 17.9	0.83 ± 0.13 FU NA	0.0	BL: 81.6 (74.3–86.9) FU: 71.9 (65.4–77.1)	NA	NA	Decrease in Ca/Vit D	7
		BP	14 (10/4)	66.1 ± 17.0	0.75 ± 0.14 FU NA	7.1	BL: 81.1 (75.6–89.6) FU: 83.4 (76.6–93.0)			Increase in TPTD + Dmab	
		TPTD	5 (1/4)	69.8 ± 8.0	0.62 ± 0.12 FU NA	60.0	BL: 70.0 (64.0–72.6) FU: 81.8 (73.3–88.9)				
		DMAb	14 (5/9)	58.9 ± 12.8	0.72 ± 0.15 FU NA	14.3	BL: 76.2 (72.0–84.9) FU: 84.0 (79.2–90.0)				
Tsai et al. (68) (Ab)	TPTD treatment, FU 3 months	TPTD 20 mg	33 (0/33)	NA (52–83)	NA	NA	BL: 82.1 ± 8.3* (61.5–102.7) FU: −4.8%	NA	NA	Decrease in TPTD 20 + 40 mg	NA
		TPTD 40 mg	29 (0/29)				BL: 83.2 ± 10.1* (67.1–99.3) FU: −7.0%				

Age is presented as mean ± SD (range, if reported), BMD is presented as mean ± SD and BMSi is presented as mean ± SD (95% CI) or median (IQR). 95% CI calculated from data provided in the publication. Ab, published only in Abstract form; BL, Baseline; BMD, Bone mineral density; BMSi, Bone material strength index; BP, Bisphosphonate; Ca, Calcium; DMAb, Denosumab; FN, Femoral neck; FU, Follow-up; Fx, Fracture; GC, Glucocorticoid; OP, Osteoporosis; TPTD, Teriparatide. *Measured in a subgroup of subjects.

TABLE 5 | Impact microindentation studies in population-based cohorts.

References	Subjects	(m/f)	Age (years)	FN BMD (g/cm²)	Fx prevalence (%)	BMSi	Relationship of BMSi with			Study quality scale (0–10)
							Age	BMD	Fx	
Rudang et al. (51)	Elderly women	211 (0/211)	78.3 ± 1.1 (75–80)	0.65 ± 0.10	55.5	75.6 ± 7.6 (74.6–76.6)	No	Pos	No	6
Sundh et al. (40)	Elderly women	202 (0/202)	78.2 ± 1.1 (75–80)	NA	NA	75.6 ± 7.6 (74.6–76.6)	NA	NA	No	8
Johansson et al. (43)	Elderly women VF	277 (0/277)	77.8 ± 1.4 (75–80)	0.64 ± 0.09*	100	76.9 ± 7.3* (75.7–78.1)	NA	NA	No	6
	Elderly women No VF	750 (0/750)	77.7 ± 1.6 (75–80)	0.67 ± 0.10*	0	77.9 ± 7.4* (77.1–78.7)				
Rufus-Membere et al. (66)	Men	252 (252/0)	63.2 ± 12.6 (33–96)	NA	NA	83.0 ± 6.4 (82.2–83.8)	No	NA	NA	NA
Rufus-Membere et al. (54)	Men	357 (357/0)	63.2 ± 13.8 (33–96)	0.96 ± 0.13	11.9	Fx: 80.2 ± 6.9 (78.0–82.4) No Fx: 82.8 ± 6.1 (82.1–83.5)	Neg	No	Pos	5

Age is presented as mean ± SD (range), BMD is presented as mean ± SD and BMSi is presented as mean ± SD (95% CI). 95% CI calculated from data provided in the publication. BMD, Bone mineral density; BMSi, Bone material strength index; FN, Femoral neck; Fx, Fracture; VF, Vertebral fracture. *Measured in a subgroup of subjects.

Impact Microindentation Studies in Secondary Osteoporosis (Table 2)

Any systemic disorder may affect the skeleton and alter the material properties of bone thus increasing fracture risk. Our literature search yielded 17 studies addressing the value of IMI in assessing fracture risk in secondary osteoporosis: eight in patients with a variety of endocrine diseases (36, 41, 48, 49, 62–65), four in patients infected with the Human Immunodeficiency Virus (HIV) (46, 47, 52, 59), three in patients with chronic kidney disease (45, 53, 58), one in patients with chronic atrophic gastritis (56) and one in patients with monoclonal gammopathy of undetermined significance (MGUS) (66).

Endocrine Disorders

Secondary osteoporosis is a common co-morbidity of endocrine disorders, resulting from direct and indirect effects of hormonal excess or deficiency on the bone remodeling cycle, bone mineral content and bone matrix composition (71). The eight published studies in patients with endocrine disorders (four in abstract form) included a total of 304 patients in whom IMI was performed in addition to standard evaluation of fracture risk using DXA. Six of these studies were performed in patients with Diabetes mellitus (one in type 1, five in type 2) (41, 48, 49, 62–64), one in Acromegaly (36), and one in parathyroid disorders (primary hyperparathyroidism and hypoparathyroidism) (65).

Diabetes mellitus (DM)

The effect of DM on the skeleton is multifactorial. The main cause of osteoporosis in DM is low bone formation with the two main contributing factors for this being a shift from osteoblastogenesis to adipogenesis, and the toxic effects of the accumulation of advanced glycation end products (AGE) on osteoblasts, both leading to the characteristic low bone turnover and increased fracture risk particularly observed in type 1 DM (T1DM). Fracture risk is however increased in both T1DM and type 2 DM (T2DM) with BMD measurements shown to underestimate fracture risk (72). This suggests that tissue material properties of bone are likely to be impaired in patients with DM and that the evaluation of BMSi may provide information on bone strength not captured by BMD. Five of the six studies in DM patients (T1DM n = 33, T2DM n = 131), also including patients with impaired fasting glucose concentrations (IFG, n = 37), compared BMSi in DM and prediabetes patients with that of non-diabetic controls (n = 655). Findings from these studies show significantly lower BMSi values in all DM patients (type 1 and 2), but not in prediabetes, compared to controls, with values ranging from 63.7 (SE 1.9) (49) to 80.6 (63) in DM patients and from 70.1 (SE 1.9) (49) to 85.7 (SE 1.6) (48) in controls. Data from a US group also show an inverse relationship between BMSi and mean glycated hemoglobin level (HbA1c) over 10 years prior to the study (r = −0.41; p = 0.026) (48), suggesting a direct negative effect

of prolonged hyperglycemia on bone material properties. This finding was also supported by a significant inverse relationship between BMSi and the duration of T2DM ($r = -0.68$, $p < 0.05$) observed in another US study (49). Data further show that BMSi is significantly inversely correlated with AGE data obtained from skin analysis as detected by autofluorescence ($r = -0.65$, $p < 0.05$) (49). In all these studies but one, LS and FN BMD were comparable between T2DM patients and controls, which was not the case in T1DM where a larger number of patients also had low bone mass. Data on fracture risk were not reported in any of the studies in patients with DM. Literature findings in DM therefore suggest that bone material properties are impaired in both T1DM and T2DM, independently of BMD. In the last of the six studies conducted in DM patients, BMSi was significantly higher in T2DM patients with higher ergonometrically measured fitness although this was only published in abstract form (62).

TABLE 6 | Impact microindentation studies in healthy subjects.

CROSS-SECTIONAL DESIGN

References	Subjects	(m/f)	Age (years)	FN BMD (g/cm²)	Fx prevalence (%)	BMSi	Relationship of BMSi with			Study quality scale (0–10)
							Age	BMD	Fx	
Duarte Sosa et al. (42)	Women from Norway	42 (0/42)	46.3 ± 13.6	1.03 ± 0.10	0	77.0 ± 7.1 (74.9–79.2)	No	No	NA	5
	Women from Spain	46 (0/46)	46.7 ± 15.4	0.83 ± 0.12	0	80.7 ± 7.8 (78.4–83.0)				
Taynouni et al. (61)	Healthy volunteers	88 (19/69)	Men: 34 (24–98) Women: 49 (30–81)	NA	NA	88.0 ± 7.6 (84.3–91.7) 82.0 ± 7.4 (80.3–83.8)	No	NA	NA	3
Guerri et al. (70) (Ab)	Subjects prior to knee replacement	10 (5/5)	72 ± 5 (59–83)	0.66 ± 0.08	NA	75.8 ± 6.0 (71.6–80.1)	NA	NA	NA	NA

LONGITUDINAL DESIGN

References	Intervention	Subjects	(m/f)	Age (years)	FN BMD (g/cm²)	Fx prevalence (%)	BMSi	Relationship of BMSi with			Study quality scale (0–10)
								Age	BMD	Fx	
Sundh et al. (40)	Exercise of one leg, FU after 3 months	Inactive women	20 (0/20)	55.5 ± 2.3 (51–59)	BL: 0.72 ± 0.08 FU: NA	NA	BL: 73.4 ± 6.8 (70.7–76.1) FU: 76.8 ± 9.0 (72.6–81.0)	NA	NA	NA	8

Age is presented as mean ± SD (range, if reported) or median (range), BMD is presented as mean ± SD and BMSi is presented as mean ± SD (95% CI). 95% CI calculated from data provided in the publication. Ab, published only in Abstract form; BL, Baseline; BMD, Bone mineral density; BMSi, Bone material strength index; FN, Femoral neck; FU, Follow-up; Fx, Fracture.

Acromegaly

Skeletal changes in acromegaly are due to GH excess and are characterized by high bone turnover in favor of increased bone formation. Although BMD is generally normal or increased, the disorder is associated with an increased risk for vertebral fractures (71). Our group showed that BMSi was significantly lower in 48 patients with acromegaly despite long-term remission: 16.1 years (range 0.5–37.8 years), compared with BMSi measurements in 44 age-matched controls, 79.4 (SE 0.7) vs. 83.2 (SE 0.7), $p < 0.001$, although LS and FN BMD were comparable between groups (36). This finding suggests that tissue material properties of bone are likely to be irreversibly altered in patients with acromegaly leading to persistent increased fracture risk despite long-term adequate control of GH excess (73). Intriguingly, we could not demonstrate a difference in BMSi between patients with ($n = 28$) or without ($n = 20$) vertebral fractures (36).

Parathyroid disorders

BMSi was measured in patients with parathyroid disorders in a single study only published in abstract form (65). Parathyroid hormone (PTH) plays an important role in the maintenance of bone mass and integrity, and both excess or decrease of the circulating hormone may potentially affect fracture risk. Whereas bone turnover is low in hypoparathyroidism, it is high in hyperparathyroidism in favor of bone resorption, resulting in bone loss particularly at cortical sites. Although both non-vertebral and vertebral fracture risk have been shown to be increased in hyperparathyroidism (74, 75), data in hypoparathyroidism are scarce and conflicting (76). Starr et al. found that BMSi was significantly lower in 13 patients with primary hyperparathyroidism than in 22 age- and sex-matched controls with normal PTH values (respectively, 67.8 ± 9.0 vs. 77.2 ± 8.0, $p < 0.05$). Interestingly, BMSi was also found to be lower in 15 patients with hypoparathyroidism compared to controls (respectively, 68.4 ± 10.0 vs. 77.2 ± 8.0, $p < 0.05$). As expected, BMD T-scores were higher in hypoparathyroidism than in hyperparathyroidism patients at all sites except at the LS. No data on fracture risk were provided (65).

Patients Infected With HIV

BMSi was measured in a total of 153 patients with HIV in four studies conducted by the Spanish group (46, 47, 52, 59). Patients infected with HIV are at increased fracture risk possibly due to the viral infection itself or its treatment, particularly when using the antiretroviral drug tenofovir disoproxil fumarate (TDF), although the exact mechanism by which this drug increases bone fragility remains elusive. In an initial study by Güerri-Fernández et al. BMSi was found to be significantly lower in 50 untreated HIV patients compared to 35 healthy HIV-negative controls [84.5 (83.0–87.0) vs. 90.0 (88.5–93.0), respectively, $p < 0.001$], in the presence of similar BMD values (46). Findings from two further studies showed a significant increase

in BMSi from 86.1 ± 6.1 to 89.0 ± 4.2 ($p < 0.001$), reaching comparative values to those of healthy controls, 12 months after starting antiretroviral therapy with TDF, while BMD values decreased on treatment (52, 59). No data are provided on the effect of these findings on fracture risk. BMSi values were not found to be different in patients on long-term treatment with TDF compared to patients using the different antiretroviral agent abacavir, BMSi 81.0 (0.8) vs. 82.7 (1.3), $p = 0.27$, respectively (47).

Chronic Kidney Disease (CKD)

Data on BMSi in CKD were retrieved from three studies conducted by the Spanish group (45, 53, 58). BMSi values were significantly lower in 35 CKD patients on dialysis compared to 94 healthy non-CKD controls [respectively, 79.0 (71.8–84.2) vs. 82.6 (77.5–88.9), $p < 0.05$] (45). In a second study, BMSi was measured cross-sectionally in 38 kidney transplant recipients more than 10 years after kidney transplantation. BMSi was found to be low in long-term transplant recipients compared to 93 younger healthy controls, although the difference was no longer observed after adjusting for age, sex, and BMI. This suggests that bone material properties may improve in kidney transplant recipients in the long-term (58). The most recent data were from a longitudinal study conducted in 14 patients undergoing kidney transplantation on a low glucocorticoid dosing protocol with follow-up BMSi performed 3 and 12 months post-transplantation. These data showed no significant change in BMSi compared to baseline values both at 3 months and at 12 months post-transplant [respectively, 80.1 (73.0–85.4) vs. 79.2 (73.2–85.4), no *p*-value provided] despite a significant transient decrease of FN BMD at month 3 and a significant decrease of LS BMD at month 12 (53).

Chronic Atrophic Gastritis (CAG)

A small study in 14 Norwegian patients with CAG and 18 age- and sex-matched healthy controls reported no difference in BMSi between patients and controls (respectively, 82.0 ± 9.6 vs. 80.0 ± 7.0, no *p*-value provided) in the presence of similar LS and FN BMD values. No fracture data were provided in this study, and the only suggested potential contributory factor to fracture risk in this condition was the chronic gastric hypoacidity (56).

Monoclonal Gammopathy of Undetermined Significance (MGUS)

BMSi was measured in a single study only published in abstract form comparing data between 22 patients with MGUS and age-matched controls, the number of whom was not provided. Despite normal and comparable BMD values between MGUS patients and controls, a significantly lower BMSi was observed in MGUS patients compared to controls (respectively, 68.3 ± 5.0 vs. 83.0 ± 4.0, $p < 0.001$) (66).

These findings suggest that impaired material properties of bone contribute to the increased fracture risk reported in MGUS (77).

Impact Microindentation Studies in Rare Metabolic Bone Disorders (Table 3)

Three studies were performed in a total of 28 patients with rare metabolic bone disorders: One in Type 1 Gaucher Disease (n = 16) (57), one in Paget's disease of bone (n = 9) (67) and one in Camurati-Engelmann disease (n = 3) (60).

Type 1 Gaucher Disease

Type 1 Gaucher Disease is a rare lysosomal lipid storage disorder due to beta-glucocerebrosidase deficiency leading to the accumulation of glucocerebroside in cells of the macrophage lineage. In this disorder the mechanism of bone fragility is multifactorial including mechanical replacement of the bone marrow by direct infiltration by Gaucher cells and the increased production of inflammatory factors such as cytokines leading to an imbalance in bone turnover in favor of bone resorption (78). The lower BMSi values measured in 16 patients with type 1 Gaucher Disease compared to those of 29 age- and sex-matched healthy volunteers (respectively, 72.7 ± 10.0 vs. 81.8 ± 1.4, $p < 0.05$) are in keeping with the changes observed at predominantly cortical skeletal sites, although LS BMD was also decreased in these patients compared to controls. The main marker of disease activity, chitotriosidase, was also found to be inversely correlated with BMSi (R^2= 0.516, $p < 0.05$), suggesting that in Gaucher disease material properties of bone are more severely altered the more severe the disease is (57).

Paget's Disease of Bone

In a study performed by our group, BMSi values were measured in 9 patients with unilateral Paget's disease of the tibia, in remission after treatment with bisphosphonates. Data were compared with BMSi values of the contralateral non-pathologic tibia. We observed no significant difference in BMSi between affected and non-affected tibia, 74.7 (SE 1.7) vs. 78.7 (SE 1.3), p = 0.12. However, we did observe a significant difference in serial indentations of pathologic and normal tibia. The variation of consecutive single indentation values was significantly greater in the affected tibia compared to the contralateral healthy tibia, suggesting heterogenous tissue material properties of the pagetic bone. This high variability in measurements may represent a potential sign of altered material bone properties in Paget's disease of bone (67).

Camurati-Engelmann Disease

In a series of three patients with Camurati-Engelmann disease, a rare bone disease characterized by progressive hyperostosis mainly of the diaphysis of long bones,

BMSi values were lower compared to BMSi values in 29 healthy controls, albeit non-significantly (76.9 vs. 81.4, $p = 0.17$), despite the characteristic cortical hyperostosis. This finding suggests that bone material properties can also be altered despite increased cortical volume (60).

Impact Microindentation Studies in Patients Receiving Osteoporosis Treatment (Table 4)

BMSi was measured in three studies in patients receiving antiosteoporotic therapy ($n = 117$) in order to evaluate whether IMI had the potential to be used in evaluating treatment-induced changes in bone fragility in osteoporosis (37, 44, 68).

In the first study, BMSi was measured in 52 patients with various underlying diseases requiring glucocorticoid therapy before starting treatment with prophylactic anti-osteoporosis therapy. There was a significant decrease in BMSi after 7 weeks of treatment in the Calcium/Vitamin D3-treated group ($n = 19$), from 81.6 (74.3–86.9) to 71.9 (65.4–77.1), $p < 0.05$, compared to no significant changes in the Calcium/Vitamin D3 and additional oral bisphosphonate-treated group ($n = 14$), 81.1 (75.6–89.6) vs. 83.4 (76.6–93.0), $p = 0.83$. In contrast, a significant increase in BMSi [76.2 (72.0–84.9) to 84.0 (79.2–90.0), $p < 0.05$] was observed in the denosumab-treated group ($n = 14$), with the largest increase observed in a teriparatide-treated group ($n = 5$) [BMSi 70.0 (64.0–72.6) to 81.8 (73.3–88.9), $p < 0.05$]. It is of note that a significant increase of BMSi to 87.7 (78.7–96.5) was eventually also demonstrated in the oral bisphosphonate-treated group but only at the 20 weeks measurement timepoint compared to baseline ($p = 0.043$). This study was the first to show a change in BMSi in response to medical treatment (44).

In a second study published as a meeting abstract, BMSi was measured in seven patients with osteoporosis before and after 3 months of treatment with daily subcutaneous teriparatide 20 mcg ($n = 3$) or 40 mcg ($n = 4$). In this study a significant decrease in BMSi from baseline (−4.8 ± 10.7%, $p = 0.011$) was observed in the three patients receiving the lower dose of 20 mcg and the decrease was larger in the four patients receiving 40 mcg a day, −7.0 ± 15.5%, $p = 0.011$ (68). The Abstract format did not allow the authors to formulate a hypothesis for this discrepant finding. A full paper has not been published.

In the third study, BMSi was measured cross-sectionally in 39 patients with osteoporosis but without fractures before receiving bisphosphonate treatment for 4–14 years. BMSi values were found to be significantly lower in 21 patients with incident fractures under treatment with bisphosphonates, compared to 18 patients who did not sustain fractures during treatment (respectively, 73.8 ± 6.5 vs. 81.6 ± 6.3, $p <$

0.05). BMD at the LS was also lower in the 21 patients who had sustained incident fractures (0.66 ± 0.1 vs. 0.82 ± 0.1, $p < 0.05$) (37).

Impact Microindentation Studies in Population-Based Cohorts (Table 5)

BMSi was measured in 489 individuals from a Swedish population-based cohort and in 357 subjects from an Australian cohort. The objective of the Swedish cohort, initiated in 2013, was to identify factors contributing to fracture risk in older women aged 75–80 years (39, 41, 43, 51). The Australian cohort was designed to investigate the epidemiology of osteoporosis in men across ages 33–96 years from the Geelong Osteoporosis study (54, 63, 69).

In the Swedish cohort, there was no difference in BMSi between fracture ($n = 117$) and non-fracture patients ($n = 63$), 76.1 ± 7.4 vs. 75.7 ± 7.9 ($p = 0.4$), also after stratification for low bone mass (51). BMSi did not also differ between women with vertebral fractures ($n = 141$) and those without ($n = 331$), 76.9 ± 7.3 vs. 77.9 ± 7.4, $p = 0.15$, nor was there an association between BMSi and number and/or severity of vertebral fractures (43). An inverse relationship was found between BMSi and the amount of subcutaneous fat at the tibia, whole body fat mass and BMI, suggesting a possible negative influence of adipose tissue on bone strength. BMSi was also found to be associated with cortical porosity as measured by HR-pQCT and cortical volumetric BMD at the distal tibia (39).

In a recent publication of data from the Australian cohort, analysis of the association between BMSi and FRAX clinical risk factors showed that BMSi was significantly lower in men with a prior fracture ($n = 38$), compared to those without ($n = 319$) (respectively, 80.2 ± 6.9 vs. 82.8 ± 6.1, $p = 0.024$), and in men with a history of parental hip fracture ($n = 34$) compared to those without ($n = 323$) (respectively, 80.1 ± 6.1 vs. 82.8 ± 6.9, $p = 0.029$). Data also showed that BMSi tended to be lower in the presence of T2DM ($n = 44$) and alcohol consumption ($n = 60$), albeit non-significantly, but not in the presence of smoking ($n = 21$) or secondary osteoporosis ($n = 44$) (54). A further study conducted in the Australian cohort addressed feasibility and tolerability of BMSi measurements in 252 consecutive individuals from the cohort. Data showed that the procedure was well accepted suggesting the potential promising use of this technique in the clinic as well as in research settings (69).

Impact Microindentation Studies in Healthy Subjects (Table 6)

BMSi measurements were performed in healthy nonosteoporotic individuals ($n = 206$) in four studies. The first study focused on ethnical differences in BMSi measurements and was performed in 42 Norwegian women, aged 46.3 ± 13.6 years, and in 46 age-matched Spanish women, none of whom had ever sustained a fracture.

BMSi was found to be significantly lower in Norwegian women compared to Spanish women (respectively, 77.0 ± 7.1 vs. 80.7 ± 7.8, $p < 0.001$), while total hip BMD was significantly higher in Norwegian than Spanish women (42). Differences in tissue-level material properties of bone might therefore partially explain geographically observed differences in fracture risk. BMSi was sequentially measured in 20 healthy nonosteoporotic postmenopausal women, aged 51–60 years, before and 3 months after starting a unilateral high impact exercise program. Findings from this study showed an increase in BMSi of 7% from 73.4 ± 5.8 to 76.8 ± 9.0 ($p = 0.03$) in the exercised leg, without concomitant changes in volumetric BMD or bone microarchitecture. The authors concluded that sequential IMI may detect improvement in bone material properties within 3 months of high-impact loading before changes in bone mass or architecture can be detected (40). Two further studies performed in non-osteoporotic individuals specifically investigated the relationship between BMSi and age in 69 women with a median age of 49 years (range 30–81 years), and 19 men with a median age of 34 years (range 24–98 years) (61), and the relationship between BMSi and BMD in 5 men and 5 women, aged 72 ± 5 years (59–83 years) (70). Data from these studies show that neither age nor BMD were associated with BMSi in non-osteoporotic individuals.

Factors Influencing BMSi

The systematic review of the literature on IMI performed for any indication revealed variable outcomes with the use of this tool depending not only on subject- or disease-related factors such as age, gender, BMI, geographical region or prevalent fractures and BMD, but also depending on technique-related factors such as experience and number of operators per study, number of indentations obtained per BMSi measured, and strategies used to assess inadequate quality of single indentations.

Subject-Related Factors

Age

Published data on the relationship between BMSi and age are conflicting. Whereas, eight studies failed to show a significant relationship between these two parameters (35, 38, 42, 47, 51, 55, 61, 69), six studies did demonstrate an inverse relationship between the two (33, 34, 36, 50, 52, 54) and only one study performed in 48 patients with acromegaly, aged 60.2 ± 11.0 years, showed a significant positive relationship between BMSi and age ($r = 0.291$, $p = 0.045$) (36). Four of the eight studies showing no significant correlation between BMSi and age included subjects with very low BMSi values (35, 38, 42, 51) or a narrow age range (75–80 years) (51). A fifth and a sixth study included heterogenous groups of postmenopausal women with atypical femoral fractures ($n = 20$), hip fractures ($n = 15$), long-term bisphosphonate use ($n = 30$), and treatment-naive controls ($n = 88$) (55), or two groups of HIV-infected patients

on long-term treatment with different types of antiretroviral agents (47). A relationship between BMSi and age could not be observed in a study designed to address this issue conducted in 88 patients: 69 women aged 49 years (range 30–81 years), and 19 men aged 34 years (range 24–98 years) (61). The last study that failed to show a relationship between BMSi and age was performed in the Australian cohort, which included 252 men with a wide age range: 33–96 years (69). However, after increasing the sample size of the cohort to 357 subjects, an inverse relationship between BMSi and age did become apparent (r = −0.13, p = 0.014) (54). Among the five other studies showing an inverse relationship between BMSi and age, three were from our group and included a total of 164 subjects, aged 61.8 ± 9.4 years (range 40–85 years; r = −0.457, p = 0.002), all of whom were evaluated for increased fracture risk (33, 34, 36). The two other studies reported similar results in 191 postmenopausal women older than 50 years of age (r = −0.15, p = 0.03) (50), albeit non-significant in 40 HIV-positive subjects, aged 38 ± 9 years (r = −0.28, p = 0.07) (52).

Gender

Six studies have directly compared BMSi values between men (n = 208) and women (n = 186) (33, 34, 36, 46, 47, 56), one of which was conducted in HIV-infected patients. A significantly higher BMSi was observed in men (n = 35) compared to women (n = 15), 85.0 [83–87] vs. 80.0 (77–83), p < 0.001. In the same study there was no difference observed in 24 HIV-negative men and 11 HIV-negative women, BMSi 92.0 [88–96] vs. 89.0 [86–93], p = 0.07 (46). There was also no gender difference in the other five studies comparing BMSi values in men and women (33, 34, 36, 47, 56).

BMI

Data from three studies show BMSi to be significantly inversely correlated with BMI (n = 559) (39, 54, 69), although this association could not be confirmed in four other studies (n = 365) (33, 34, 36, 55).

Geographical variation

One study specifically addressed geographical variation in BMSi and significant differences were observed between different countries, with healthy Norwegian women having lower BMSi than healthy Spanish women (42). No other study addressed geographical variation in BMSi.

Fracture-Related Factors

BMD and fractures

Six studies reported significantly lower BMD values at one or more sites in patients with fractures compared to those without (35, 37, 38, 43, 50, 51). One study found a relationship between BMD and hip fractures but not atypical femoral fractures (55),

while five studies found no significant difference in BMD between patients with or without fractures (33, 34, 45, 54, 58). Whereas the majority of reported studies (*n* = 14) did not elicit a significant association between BMSi values and BMD measurements (33–38, 42, 45, 47, 49, 54, 55, 58, 70), three did observe a weak correlation between the two measurements (50–52).

BMSi and fractures

Six studies found significantly lower BMSi values in patients with low bone mass and fragility or stress fractures, compared to non-fracture controls (33–35, 37, 38, 54). One study reported a significant relationship between BMSi and distal radius fractures, but not hip fractures (50). However, seven other studies did not observe a significant relationship between BMSi and fractures. These were three studies in patients with a diagnosis other than osteoporosis (36, 45, 58), three epidemiological studies from the Swedish cohort including patients with fractures irrespective of trauma type (39, 43, 51), and a study including 15 patients with the rare atypical femoral fractures and 20 patients with hip fractures (55).

Technique-Related Factors

As mentioned above, variability of data collected might also be due to technique-related factors such as experience of the operator, number of operators performing the technique per study, number of indentations obtained per BMSi evaluation and strategies used to assess false single indentations.

Operator-related factors

Literature data about operator training and experience are scarce and only provided in eight of the 38 publications yielded by our search (33, 43, 48, 50, 52, 54, 61, 69). Data on intraobserver coefficient of variation (CV) provided in 16 of the 38 publications (33–36, 38–43, 46, 48, 49, 51, 52, 67) report variations ranging from 1.65% (48) to 9.1% (42). Nineteen studies provided information about the number of operators performing the IMI measurements, ranging from one to nine different operators per study (33–35, 37–40, 42–44, 49–52, 54, 55, 61, 67, 69). Data on interobserver variability were however scarce, only reported in five of the 19 studies (39, 40, 43, 51, 61), with four of the five studies being from the same group reporting an interobserver CV of 5.2% for which data were adjusted before analysis (39, 40, 43, 51). The fifth study reported an interobserver CV of "<5%" (61).

Measurement-related factors

BMSi, the outcome parameter of IMI, is a dimensionless value calculated by the OsteoProbe® software from the mean of repeated indentations on the tibia, normalized to the mean of indentations performed on the phantom PMMA material. Although 13 studies (37, 45, 47, 50, 52–55, 57, 60, 61, 67, 69) stated that they performed the IMI

measurements according to the standard operating procedure published in 2016 (8), the number of indentations obtained on the tibia, which was provided by all 31 full publications, varied widely from 5 up to 25 indentations. In contrast, the number of indentations performed on PMMA material provided by the majority of studies (*n* = 28) was consistently five as per standard protocol (33–35, 37–41, 43–55, 57–61, 67, 69). Since the older software does not automatically flag inadequate indentations, the evaluation of the adequacy of an indentation was entirely left to the judgement of the operator. Data on how inadequate indentations were identified, and methods used to delete these indentations, were not available for seven of the full publications (39, 42, 43, 48, 56, 58) and when reported, differed between studies (33–38, 40, 41, 44–47, 49–55, 57, 59–61, 67, 69).

Tolerability and Safety

Fourteen publications reported that the microindentation investigation using the OsteoProbe® was well tolerated and not associated with any major complications (35, 37, 38, 42, 44, 46–48, 51, 52, 54, 57, 60, 69). Only two minor complications were reported, a mild skin infection and a mild allergic reaction to the local anesthetic, both of which readily responded to treatment.

DISCUSSION

Identifying the patient at increased risk for a fragility fracture may be challenging, particularly in patients with secondary factors for osteoporosis, as bone mineral density measurements using DXA have been shown to underestimate true fracture risk in these patients (71, 72, 79–81). Over the past decade, evidence has been accumulating about the value of impact microindentation in the *in vivo* assessment of tissue-level material properties of bone, an important contributor to bone strength in addition to that of BMD. The number of studies addressing the value of bone material strength index (BMSi) measurements in the evaluation of fracture risk has been steadily increasing, but outcomes are not always concordant. The main aim of this systematic review of the literature was to examine the added value of impact microindentation in the evaluation of fracture risk in clinical practice. Our search yielded 38 studies that were published over the past 5 years including 19 studies published in the first 3 years and reported in a previous review (9). Data from these 38 studies highlight the ability of IMI in identifying patients with increased bone fragility, be it patients with primary osteoporosis and fractures, or patients with or at risk for secondary osteoporosis due to a variety of underlying systemic disorders including endocrine disorders such as diabetes mellitus or acromegaly. Data on the value of IMI in the follow-up of patients with increased fracture risk remain scarce but do suggest that the technique is also able to detect changes in BMSi following

bone-modifying therapy in both in primary and secondary osteoporosis. The scarce data published in patients with rare metabolic bone disorders such as Type 1 Gaucher Disease or Camurati-Engelmann disease also provide valuable insights into the relationship between tissue-level material properties of bone and fracture risk. The evaluation of BMSi is of particular interest in patients who have sustained a fragility fracture in the presence of osteopenia or normal BMD values, where DXA BMD measurements underestimate fracture risk. More than 50% of fragility fractures have been found to occur in patients with osteopenia (1), the majority of whom are currently not being offered treatment with bone-modifying agents according to most nationwide adopted treatment protocols. Studies in primary osteoporosis show that IMI could identify patients with fragility fractures, also in the subgroup of those with osteopenia (33, 38), suggesting that tissue material properties of bone are altered in patients who have sustained a fragility fracture and that IMI might therefore help in identifying patients with primary osteoporosis at increased fracture risk where DXA fails to do so. Although BMSi is measured at a cortical site, most studies are concordant in showing that low BMSi is associated with increased bone fragility at all relevant skeletal sites, vertebral, non-vertebral and hip sites (34, 35, 50). Studies that did not elicit a relationship between BMSi and fractures were either performed in small subgroups of patients with secondary osteoporosis (36, 45, 58), or in studies including patients with the lowest reported BMD values among all studies (43, 51). These findings suggest that BMSi may not be of added value in evaluating bone fragility in the presence of severely decreased bone mass which is highly predictive of high fracture risk. On the other hand, findings also suggest that in patients with very low BMSi values and high fracture risk due to impaired tissue material properties of bone, bone mass may be of less important contribution to bone fragility, as observed in studies investigating BMSi and BMD in type 2 diabetes mellitus patients (41, 48, 63). In keeping with this hypothesis, BMSi was indeed found to be low in almost all studies including patient groups with a variety of underlying systemic disorders associated with secondary osteoporosis, where bone quality rather than bone quantity is likely to have the most impact on fracture risk. It is of note that BMSi was also found to be inversely correlated with markers of underlying disease activity in secondary osteoporosis such as AGE accumulation in type 2 diabetes mellitus (49), and serum chitotriosidase levels in type 1 Gaucher Disease (57), suggesting that material properties of bone are more severely altered the more severely uncontrolled the disease is. Since BMSi values have been found to be independent of BMD values in almost all studies, findings from this systematic review strongly suggest that IMI captures elements of bone fragility not captured by BMD measurements. However, although IMI measurements have been shown to identify patients at increased risk for fracture, it has not fully been elucidated which specific mechanical properties of bone are captured by IMI. A recent study from our group addresses this issue for the first time by simultaneously evaluating tissue material properties of bone by mea-

suring BMSi and bone composition of trans-iliac bone biopsies in humans *in vivo* in 12 patients with a variety of metabolic bone disorders and variable fracture risk (five patients with osteopenia and fractures, four patients with secondary osteoporosis, and three patients with rare bone diseases). The demonstration of both a negative correlation between BMSi and cortical porosity at the micro- and nanolevel and of a positive correlation of BMSi with the bone organic matrix parameters glycosaminoglycan and pyridinoline, and with mineral parameters, suggests that BMSi is affected by the composition of bone organic matrix and by bone mineral properties (82). The IMI technique has therefore the potential to be used as an additional tool to DXA in the evaluation, and also probably follow-up, of bone fragility in the clinic since sequential BMSi measurements have been shown to increase in situations associated with a decrease in fracture risk (44, 52, 59), and to decline where fracture risk increases, such as observed in patients starting glucocorticoids (44). Notwithstanding, this systematic review of the literature encountered a number of limitations in the interpretation of the generated data. One of these limitations was the high variability in the methodological quality of the published studies. Although on the whole study quality was judged to be satisfactory, quality scores ranged widely from 1 to 10 out of 10 possible points using the adapted Newcastle-Ottawa Scale. Most studies included small numbers of patients and were performed in selected patient groups, potentially resulting in a selection bias. It should also be emphasized that none of the published studies on IMI had longitudinal fracture data and the majority had a cross-sectional design except for six studies with sequential BMSi measurements under an intervention with either bone-modifying agents or exercise. Although low BMSi as measured by IMI has been clearly linked with the presence of any type of fracture, the limitations attached to a number of the studies addressing this issue do not permit, so far, to extrapolate that BMSi measurements may be predictive for increased future fracture risk. In addition, although tissue material properties of bone as measured by IMI have been shown to be altered in almost all studies investigating patient groups with secondary osteoporosis, only three of these studies compared BMSi values of fractured patients with those of nonfractured ones. No significant difference in BMSi values was observed between patients with and without fractures, although subgroups were small.

Another important limitation encountered in the interpretation of IMI data generated by our systematic literature review is the high variability of BMSi values observed in the control arm subjects between studies. Some studies reported BMSi values of 81 or higher in control subjects, whilst others reported lower BMSi values in the range of 70–78, corresponding to the range of BMSi values observed in patient groups with increased fracture risk; **Tables 1**–**6**. Our systematic review has identified a number of potential subject-, disease- and technique-related factors for this discrepancy. Our and other groups consistently reported a significant relationship between BMSi

and age, with a significant decrease in BMSi observed with increasing age. This indicates that tissue material properties of bone as measured with IMI do decline with age, which would also be expected given the increasing fracture risk associated with aging. The absence of an observed relationship between BMSi and age in some studies may possibly be due to a narrow age range (46, 51), a small number of patients included (61), or the inclusion of heterogenous groups of patients (55). In addition, studies that did not observe a relationship between BMSi and age report some of the lowest BMSi values measured (35, 38, 42, 51). Geographical differences might also influence BMSi values as observed in a study specifically designed to address this issue (42), and this is supported by the observation of this review that non-fractured elderly women from Sweden had BMSi values below 78 (39, 43, 51), which were remarkably lower than BMSi values of non-fractured controls from The Netherlands (33, 34) and Spain, who had BMSi values above 81 (37, 45, 46, 57, 58, 60, 61). Different BMSi values of subjects from different geographical regions could reflect their difference in fracture risk and this should be taken into account when comparing studies. The only currently available age-dependent reference values are those suggested by the manufacturer (Active Life Scientific) and these remain to be confirmed. Another complicating issue in the interpretation of BMSi results is that currently used IMI protocols vary in a number of key points, which hampers pooling of data and general application of the technique and may partly explain the variation in BMSi outcome between studies. The OsteoProbe® is a relatively easy to use handheld device specifically designed for *in vivo* use in humans, but it is also prone to variability in its results, which is at least partly due to the lack of a standard operating procedure until the recently published technical recommendations by Diez-Perez et al. (8). However, studies still use different protocols even after this publication. Part of the identified technique-related aspects of IMI will be addressed by the introduction of an updated system in which the software will automatically discard measurements that are more than two standard deviations away from the subject's mean, although large deviations might reflect bone disease (67). Of additional importance is adequate operator training and limitation of the number of operators per study, to minimize intra- and interobserver variability. Only after these methodological inconsistencies are addressed and future measurements are strictly conducted according to the standard protocol can normative values be obtained, and data compared between studies and centers.

Taken together, findings from this systematic review of the literature show that impact microindentation is a promising technique enabling physicians to evaluate *in vivo* tissue-level material properties of bone in a minimally invasive, simple and safe manner. Data generated by impact microindentation have contributed to the better understanding of factors involved in the pathogenesis of bone fragility. Data have also been shown to be valuable not only in the evaluation of bone fragility, but pos-

sibly also in the follow-up, particularly of patients with potentially underestimated fracture risk. BMSi is not a measure of bone mass, and no clear relationship has been demonstrated between the two, implying that IMI could be used as an additional tool to DXA BMD in the assessment of bone health rather than as a replacement of the latter in the individual patient. However, the value of IMI in predicting future fractures and treatment outcomes has yet to be established in prospective multicenter trials using standard operating procedures before recommending the routine use of the technique in the clinic.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo. 2020.00015/full#supplementary-material

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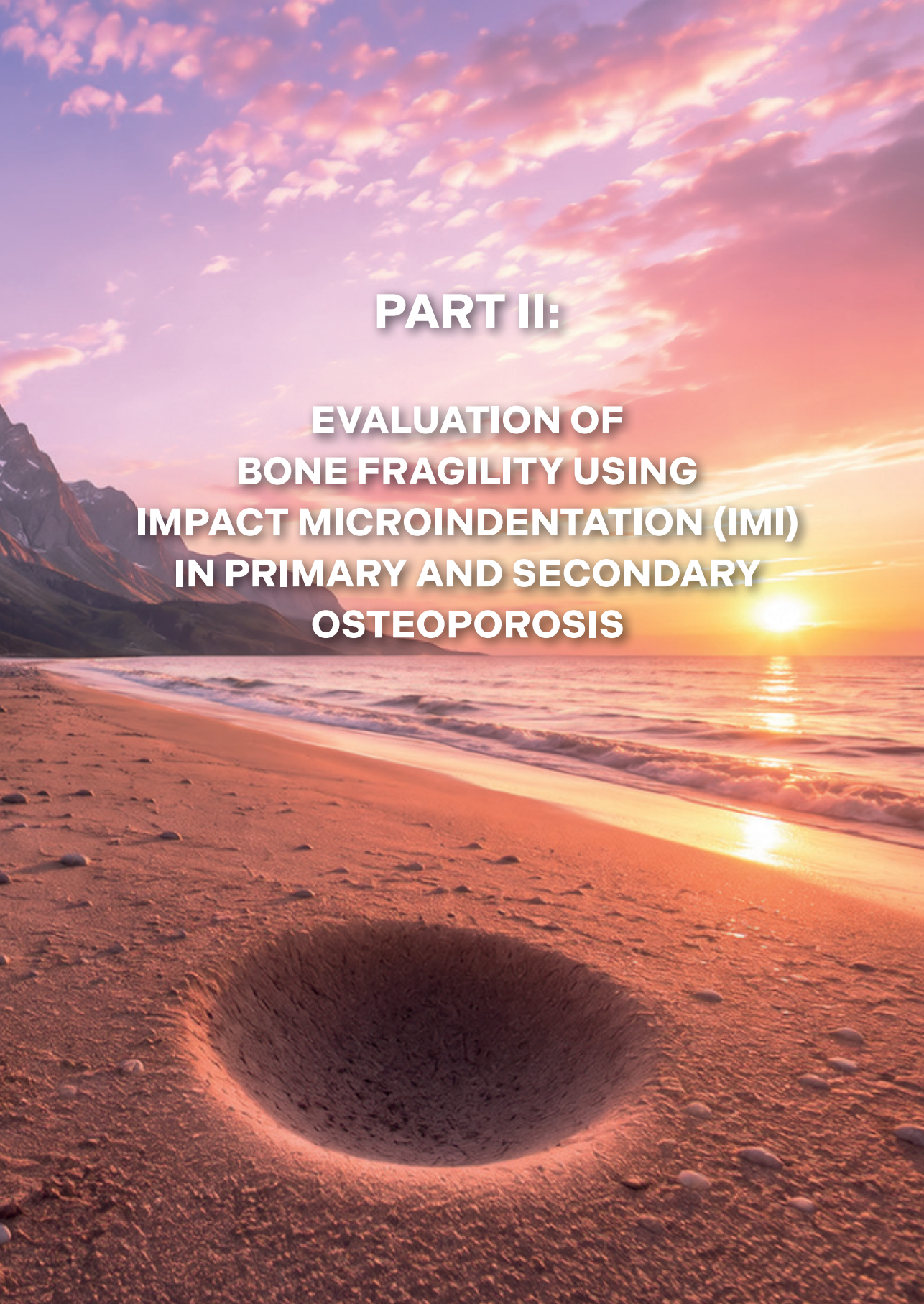

PART II:

EVALUATION OF BONE FRAGILITY USING IMPACT MICROINDENTATION (IMI) IN PRIMARY AND SECONDARY OSTEOPOROSIS

Chapter 3

Bone material strength index as measured by in vivo impact microindentation is normal in subjects with high-energy trauma fractures

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Abstract

Summary Bone material properties were assessed using impact microindentation in patients with high-energy trauma fractures. Compared to patients with low-energy trauma fractures, bone material strength index was significantly higher in patients with high-energy trauma fractures, and did not differ between patients with osteopenia and those with osteoporosis within each trauma group.

Introduction Impact microindentation (IMI) is a technique to assess tissue-level properties of bone at the tibia. Bone material strength index (BMSi), measured by IMI, is decreased in patients with low-energy trauma fractures, independently of areal bone mineral density (aBMD), but there is no information about BMSi in patients with high-energy trauma fractures. In the present study, we evaluated tissue-level properties of bone with IMI in patients with high-energy trauma fractures.

Methods BMSi was measured 3.0 months (IQR 2.0–5.8) after the fracture in 40 patients with high-energy trauma and 40 age- and gender-matched controls with low-energy trauma fractures using the OsteoProbe® device.

Results Mean age of high- and low-energy trauma patients was 57.7 ± 9.1 and 57.2 ± 7.7 years, respectively ($p = 0.78$). Fracture types were comparable in high- vs low-energy trauma patients. Lumbar spine (LS)-aBMD, but not femoral neck (FN)-aBMD, was higher in high- than in low-energy trauma patients (LS 0.96 ± 0.13 vs 0.89 ± 0.13 g/cm2, $p = 0.02$; FN 0.75 ± 0.09 vs 0.72 ± 0.09 g/cm2, $p = 0.09$). BMSi was significantly higher in high- than in low-energy trauma patients (84.4 ± 5.0 vs 78.0 ± 4.6, $p = 0.001$), also after adjusting for aBMD ($p = 0.003$). In addition, BMSi did not differ between patients with osteopenia and those with osteoporosis within each trauma group.

Conclusion Our data demonstrate that BMSi and LS-aBMD, but not FN-aBMD, are significantly higher in high-energy trauma patients compared to matched controls with similar fractures from low-energy trauma. Further studies of nonosteoporotic patients with high-energy trauma fracture with measurements of BMSi are warranted to determine whether IMI might help in identifying those with reduced bone strength.

Introduction

Fractures following severe physical trauma are typically excluded from most studies of the prevalence or the incidence of osteoporotic fractures or from efficacy outcomes in clinical intervention trials of subjects at increased risk for fractures [1–3]. However, observational studies reported that subjects with prior high-energy trauma fractures had similar areal bone mineral density (aBMD) values as subjects with prior low-energy trauma fractures and lower aBMD values than subjects without fractures [4, 5]. This raised the possibility of a common, aBMD-related, pathogenetic mechanism of high- and low-energy trauma fractures. It was recommended that classification of fractures as high or low-trauma and fragility — or any other terms that suggest a rating of trauma — should be abandoned, and all types of fractures should be evaluated and treated without regard to trauma [5, 6].

In an analysis of two large prospective cohorts of women and men [6], the risk of a future high-energy trauma fracture was increased only among individuals with osteoporosis (baseline aBMD T-score ≤ −2.5 SD) but not among women or men with osteopenia (baseline aBMD T-score between −1.0 SD and −2.5 SD), and the rate of future high-energy trauma fractures was considerably lower than that of low-energy trauma fractures. In addition, high-energy trauma fractures were, contrary to low-energy trauma fractures, not associated with increasing age or mortality [6]. These findings suggest that determinants of bone fragility other than aBMD may be different between subgroups of subjects with high-energy trauma fractures and also between subjects with high- and subjects with low-energy trauma fractures.

Fractures occur when the loads applied to bone exceed its strength which is determined by the amount of bone (i.e., size or mass), the spatial distribution of bone mass (i.e., shape and architecture), and the intrinsic properties of the materials that comprise the bone [7]. Bone microarchitecture (both cortical and trabecular) can be assessed in vivo by high-resolution peripheral computed tomography (HRpQCT) [8]. A recent large, prospective study of women and men at least 40 years of age reported that indices measured by HR-pQCT improved prediction of fractures beyond femoral neck aBMD and the fracture risk assessment tool FRAX [9]. Fractures included in this report were, however, mostly low-energy trauma (67%), but traumatic fractures (19%) were also included.

Bone material properties are the least understood and hardest to evaluate determinant of bone strength. Impact microindentation (IMI) is a relatively new reference point indentation technique that measures tissue-level properties of bone in humans in vivo [10]. IMI is performed with the handheld device OsteoProbe® that imparts a single impact load to the bone surface and is approved for use at the tibia. By driving

the probe into the bone surface, the resistance of bone tissue to a given mechanical challenge can be measured as bone material strength index (BMSi) [11]. Although measured at a cortical site, low BMSi was associated with increased bone fragility at all relevant skeletal sites, vertebral, non-vertebral, and hip sites, in individuals with low-energy trauma fractures [12–14]. There is, however, no information about bone material properties in patients with high-energy trauma fractures. The aim of this study was to evaluate bone material properties, measured by IMI, in subjects with high-energy trauma fractures to test the hypothesis that BMSi might differ between subjects with high- and subjects with low-energy trauma fractures.

Patients and methods

Patients

This was a cross-sectional study of women and men, aged 40 to 85 years, with high-energy trauma fractures recruited from the fracture liaison service (FLS) and the outpatient clinic of the Center for Bone Quality of the Leiden University Medical Center who consented to measurement of BMSi by OsteoProbe®. Subjects were matched for gender and age with patients with low-energy trauma fragility fractures investigated in the same center during the same period who served as controls; aBMD, measured by dual-energy X-ray absorptiometry (DXA), was not a selection criterion in either group of subjects with fractures. Exclusion criteria for both groups were a metabolic bone disease other than osteoporosis, use of any treatment affecting bone metabolism except calcium and vitamin D, immobilization, and inability to provide informed consent. Patients were also excluded if there was a contraindication for IMI measurement (systemic infection, severe obesity, or allergy to local anesthetic used). If there was local skin or bone lesions of the tibia, prior fracture of the tibia or local edema, the contralateral tibia, if free of problems, was used [15]. The study was approved by the Medical Ethics Committee of the LUMC, and written informed consent was obtained from all study participants.

Methods

Full medical history, clinical risk factors for fracture, and details of the conditions associated with the presenting fracture were documented in all studied subjects. A high-energy trauma fracture was defined as any fracture caused by major trauma (e.g., fall from height > 2 m, motor vehicle accidents, high-speed biking, and skiing injuries). A low-energy trauma fracture was defined as any fracture following a fall from standing height or less. All fractures were radiographically confirmed; of the vertebral fractures, only grade 2 or higher [16] was considered.

The 10-year probability (FRAX) for a major osteoporotic fracture and for a hip fracture was calculated using reference values for the Dutch population [17]. Presenting fractures were not included as previous fractures in the calculation of the FRAX.

Laboratory measurements

Serum calcium (albumin-corrected) and creatinine concentrations and alkaline phosphatase activity were measured by semiautomated techniques. Plasma intact parathyroid hormone (PTH) was measured by the IMMULITE 2500 (Siemens Diagnostics, Breda, the Netherlands) and serum 25-hydroxyvitamin D (25-OH D) concentrations by the 25-OH-vitamin D TOTAL assay (DiaSorin D.A./N.V., Brussels, Belgium).

Bone mineral density

Areal BMD was measured at the lumbar spine (L1-L4) and at the left and right hip by DXA with Hologic QDR Discovery A (Hologic, Bedford, MA, USA). Average aBMD values of the hip were used in the analysis. NHANES III reference values compatible with reference values of the Dutch population were used to calculate T-scores [18]. Osteopenia and osteoporosis were diagnosed according to the World Health Organisation (WHO) criteria with osteopenia defined as a T-score of < -1.0 SD but > -2.5 SD at either the lumbar spine or femoral neck and osteoporosis as a T-score of ≤ -2.5 SD below the young female adult mean [19].

Impact microindentation

Bone material strength index was measured in all patients by impact microindentation using the handheld microindenter device (OsteoProbe® RUO, Active Life Scientific, CA, USA) on the midshaft of the tibia as previously described [20]. In brief, the patient was placed in supine position with the tibia in external rotation to orient the flat surface of the medial tibia diaphysis in a horizontal position. The measurement site was defined as the mean distance between the medial malleolus and the distal apex of the patella. Following disinfection of the area and local anesthesia of the skin and periosteum with lidocaine 1%, the test probe was gently inserted in the skin (without skin incision) until the bone surface was reached. Usually, one skin piercing is enough to perform all indentations without pulling the indenter out of the skin. In the very rare occasion that the skin is tough (for instance in young individuals), a second skin piercing may be necessary to prevent the probe from suffering lateral tension by the skin itself that might interfere with the measurement. Each time the skin is pierced, the new first measurement is discarded. After at least five adequate measurements, five additional measurements were performed on a polymethylmethacrylate (PMMA) calibration phantom [12, 20–23]. BMSi was calculated by the corresponding computer software. Measurements were performed by three experienced operators. The intra-observer and interobserver coefficient of variation (CV) was 2.2% and 1.6%, respectively.

Statistical analysis

Data were tested for normality using Shapiro–Wilk test and visually with histograms. A paired *t*-test was used to test between-group differences in BMSi and aBMD. Between-group differences in baseline characteristics were assessed using a paired *t*-test or Wilcoxon signed-rank test and McNemar's test for normally and not normally distributed continuous and for categorical variables, respectively. Correlations between BMSi and aBMD values or patients' parameters were examined by Pearson's and Spearman's tests for normally and not normally distributed variables, respectively. Conditional logistic regression was used to assess BMSi values adjusted for aBMD to compare BMSi values between high- and low-energy trauma patients. Analysis of variance models with BMSi as outcome variable adjusted for age were used to compare BMSi values between high-energy trauma patients with different DXA diagnoses (normal aBMD, osteopenia, and osteoporosis). Assuming a standard deviation of 5.0 and a difference in BMSi of 3.5 based on an earlier study from our group in patients with and without fragility fractures [12], the sample size to detect a clinically significant difference in BMSi between groups with a power of 0.8 at a significance level of 0.05 is 33. A p-value < 0.05 was considered to be statistically significant. All analyses were performed using SPSS software for Windows (version 25.0; SPSS Inc., Chicago, IL, USA), and graphs were constructed with GraphPad Prism (version 8.0; GraphPad software Inc., La Jolla, CA, USA).

Results

Forty patients (70.0% men) with high-energy trauma fractures and 40 control patients (70.0% men) with low-energy trauma fractures were included in the study. Median time to inclusion after sustaining a high- or a low-energy trauma fracture was comparable between both groups [3.0 months (IQR (interquartile range) 2.0–5.8 months) and 3.0 months (IQR 1.0–5.8 months), respectively ($p = 0.899$)]. Mechanisms of injury in high-energy trauma patients were highspeed biking injury ($n = 14$), fall from height > 2 m ($n = 11$), car crash ($n = 9$), and skiing injury ($n = 5$); one patient fractured his forearm by a heavy industrial machine. Patients' characteristics and laboratory measurements are shown in Table 1. By design, there were no differences in age and gender between the two groups, and BMI values were comparable. Multiple fractures were more common in high-energy trauma patients ($n = 16$ versus $n = 5$ in low-energy trauma patients, $p = 0.005$), but the sites of fracture in individual patients were similar: clinical vertebral fracture, 10 high- versus 11 low-trauma patients, $p = 0.799$; hip fracture, 3 high- versus 4 low-trauma patients, $p = 1.00$; and non-hip non-vertebral fracture, 31 high- versus 25 low-trauma patients, $p = 0.143$.

Table 1 Characteristics of patients with high- and low-energy trauma fractures

Characteristic	High-energy trauma (n= 40)	Low-energy trauma (n= 40)	p-value
Age, y	57.7 ± 9.1 (range 40–72)	57.2 ± 7.7 (range 41–73)	0.781
Male/female (%)	28/12 (70.0/30.0)	28/12 (70.0/30.0)	1.00
BMI, kg/m^2	25.4 ± 3.4	25.3 ± 3.1	0.854
Smoking, *n* (%)	5 (12.5)	9 (22.5)	0.260
Alcohol >3 U/d, *n* (%)	4 (10.0)	3 (7.5)	0.712
Previous fracture, *n* (%)	19 (47.5%)	23 (57.5)	0.370
Presenting fracture			
> 1 fracture, *n* (%)	16 (40.0)	5 (12.5)	**0.005**
Vertebral fracture, *n* (%)*	10 (25.0)	11 (27.5)	0.799
Hip fracture, *n* (%)	3 (7.5)	4 (10.0)	1.00
NHNV fracture, *n* (%)	31 (77.5)	25 (60.0)	0.143
Laboratory parameters			
Calcium[a], mmol/L	2.32 ± 0.07	2.30 ± 0.06	0.204
Creatinine[b], umol/L	78.2 ± 13.7	76.8 ± 11.9	0.633
25-OH D[c], nmol/L	71.0 ± 25.0	65.2 ± 27.3	0.323
PTH[d], pmol/L	3.6± 0.3	3.0 ± 0.3	0.084
BMD and FRAX probabilities			
FRAX major fracture, %	3.8 ± 0.4	5.1 ± 0.5	0.201
FRAX hip fracture, %	0.7 ± 0.2	0.9 ±0.3	0.130
LS-aBMD, g/cm^2	0.96 ± 0.13	0.89 ± 0.13	**0.013**
T-score LS	− 1.1 ± 1.2	− 1.8± 1.1	**0.007**
FN-aBMD, g/cm^2	0.75 ± 0.09	0.72 ± 0.09	0.090
T-score FN	− 1.2 ± 0.7	− 1.5± 0.7	0.100

Values are expressed as mean ± SD, FRAX and PTH are expressed as median ± SEM. *aBMD*, areal bone mineral density; *FN*, femoral neck; *LS*, lumbar spine; *NHNV*, non-hip non-vertebral. *Additional VF detected on radiographs: $n = 1$ vs $n = 5$ ($p = 0.201$). [a]Calcium (albumin-corrected) reference range, 2.15–2.55 mmol/L. [b]Creatinine reference range, 64–104 umol/L for males; 49–90 umol/L for females. [c]25-OH vit D reference range, 50–250 nmol/L. [d]PTH reference range, 0.7–8.0 pmol/L

Lumbar spine aBMD (LS-aBMD), but not femoral neck aBMD (FN-aBMD), was significantly higher in high-energy trauma patients (Table 1). In this group, 10 patients (25%) had normal aBMD values, 23 (57.5%) had osteopenia, and 7 (17.5%) had osteoporosis. The corresponding numbers in the low-energy trauma group were 3 (7.5%), 23 (57.5%), and 14 (35.0%). Thus, significantly more patients in the high-energy trauma group had normal aBMD values ($p = 0.047$), but the number of patients with a diagnosis of osteoporosis/osteopenia did not differ between the two groups (osteoporosis $p = 0.075$, osteopenia $p = 1.00$).

BMSi values of all patients studied were not different between men and women (81.1 ± 6.3 vs 81.3 ± 4.2, $p = 0.932$) but did negatively correlate with FRAX (without

aBMD) for both major and a hip fracture ($r = -0.257$, $p = 0.022$, and $r = -0.308$, $p = 0.006$, respectively) and with age ($r = -0.277$, $p = 0.013$; $r = -0.362$, $p = 0.022$ in high-energy trauma patients only). There was no significant relationship between BMSi values and LS-aBMD ($r = 0.093$, $p = 0.413$; $r = -0.269$, $p = 0.097$ in high-energy trauma patients only), FN-aBMD ($r = 0.079$, $p = 0.489$; $r = 0.045$, $p = 0.781$ in high-energy trauma patients only), or BMI ($r = -0.205$, $p = 0.068$). High-energy trauma patients had significantly higher BMSi values compared with low-energy trauma patients, 84.4 ± 5.0 versus 78.0 ± 4.6, $p = 0.001$ (Fig. 1A), also after adjusting for aBMD ($p = 0.003$). BMSi values did not differ between patients with osteopenia and those with osteoporosis within high-energy trauma patients (Fig. 1B). In addition, BMSi values did not differ between patients with osteopenia and those with osteoporosis within low-energy trauma patients, being constantly lower in low- than in high-energy trauma patients (Table 2), also after adjusting for age (osteopenia $p < 0.001$, osteoporosis $p = 0.002$). In the high-energy trauma group, there was no difference in BMSi values between patients with multiple fractures (range 2 to 13 fractures, $n = 16$) and those with only one fracture ($n = 24$), 84.4 ± 4.8 versus 85.0 ± 5.1, $p = 0.733$. In the low-energy trauma group, the number of patients with multiple fractures was too small for a similar statistical analysis.

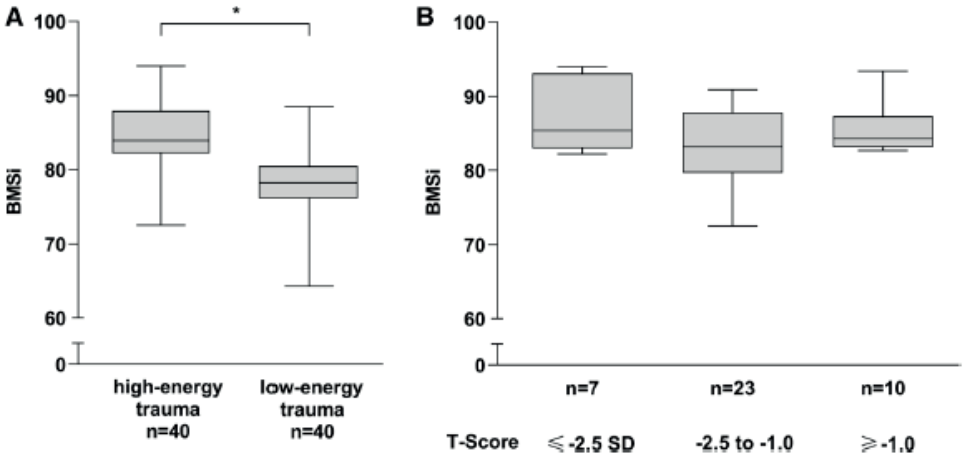

Fig. 1 Bone material strength index (BMSi) in **A** patients with high-energy trauma and low-energy trauma fractures and **B** in high-energy trauma patients with a dual-energy x-ray absorptiometry diagnosis of osteoporosis, osteopenia, and normal aBMD. Data are shown in box-whisker plots, and statistical differences are displayed for BMSi. Boxes indicate median and interquartile range. Bars indicate minimum and maximum values. *$p = 0.001$

Table 2 Characteristics of patients with osteoporosis and osteopenia and fractures according to mechanism of injury

Characteristic	Osteopenia		Osteoporosis	
	High-energy trauma (n= 23)	Low-energy trauma (n= 23)	High-energy trauma (n= 7)	Low-energy trauma (n=14)
Age, y	58.3 ± 9.2	55.9 ± 7.4	57.7 ± 10.5	58.4 ± 7.8
BMI, kg/m^2	25.8 ± 3.8	25.5 ± 3.2	22.8 ± 2.4	24.6 ± 2.9
Male/female	17/6	14/9	6/1	11/3
FRAX major fracture, %	4.3 ± 0.5	5.2 ± 0.7	3.8 ± 1.4	5.1 ± 1.0
FRAX hip fracture, %	0.8 ± 0.1	0.8 ± 0.4	1.1 ± 0.7	1.4 ± 0.6
T-score LS	− 0.9 ± 0.8[a]	− 1.3 ± 0.7	− 3.0 ± 0.5	− 3.0 ± 0.5
T-score FN	− 1.5 ± 0.4	− 1.5 ± 0.7	− 1.9 ± 0.6	− 1.8 ± 0.7
BMSi	**83.0 ± 5.2**[b]	**77.8 ± 2.8**	**87.2 ± 4.8**	**77.4 ± 6.3**

Values are expressed as mean ± SD, FRAX is expressed as median ± SEM. *BMSi*, bone material strength index; *FN*, femoral neck; *LS*, lumbar spine. [a]$p = 0.053$ compared with low-energy trauma patients with osteopenia. [b]$p < 0.001$ compared with low-energy trauma patients with osteopenia

Discussion

We show that BMSi values, measured by IMI, are significantly higher in subjects with high-energy trauma fractures than in gender- and age-matched subjects with low-energy trauma fractures and comparable with those previously reported in subjects without fracture [12, 21]. The design and size of our study allowed precise evaluation of the severity of injury, and we included only subjects with documented severe trauma in the high-energy trauma group; notably, aBMD was not a selection criterion or a prerequisite for inclusion in the study. More men than women were studied, but there was no difference in BMSi values between males and females. Moreover, the sites of fractures, including the spine and the hip, were similar between subjects with high- and low-energy trauma, but multiple fractures were more common in subjects with high-energy trauma.

To date, this is the first study that addresses the question of the effects of loads on bone material properties and fragility in humans in vivo by evaluating BMSi in subjects with high-energy trauma fractures and comparing them to those of subjects with low-energy trauma fractures. Studies comparing BMSi values between subjects with low-energy trauma fractures and subjects without fractures have generally shown significantly lower values in the former [12, 13, 21, 24]. Different from these observations, however, a Swedish cohort of women aged 75 to 80 years reported similar BMSi values in women with and without fractures [25]. In that study, all fractures irrespective of trauma type were included, and information about fractures

was obtained retrospectively. In view of the results of the present study, inclusion of subjects with high-energy trauma fractures, and presumably higher BMSi values, may have contributed to the lack of difference in BMSi between the two groups in that analysis.

The finding that BMSi values were not decreased in most subjects with high-energy trauma fractures raises questions about the mechanism of fracture in such subjects. Fractures occur when the load applied to bone exceeds its strength. The major measurable determinants of bone strength in humans in vivo are the amount of bone mineral and mass, the bone size, geometry and architecture, and the bone material composition [26]. Recent studies of the relationship between BMSi values and bone material properties that affect bone strength — measured by microspectroscopy (Raman, FITRI) in bone biopsies obtained concurrently with BMSi measurements — showed that BMSi assesses material properties of cortical bone [27, 28]. BMSi values are strongly associated with the mineral to matrix ratio (MM), the most widely measured bone material property, that, differently from other measurements of mineral density, directly measures and accounts for the amount of organic matrix in the volume analyzed [29]. In rodents, MM correlates with ash weight and is directly proportional to bending stiffness and failure moment being superior to total mineral density, measured by micro CT, in predicting bone strength [30, 31]. As reported here, material properties of cortical bone are negatively related with age and the 10-year fracture probability assessed by FRAX (without aBMD) [32]. Thus, our results showing that bone tissue properties are not impaired in subjects with fractures due to high-energy trauma suggest that the excessive load applied to bone is likely responsible for most of the fractures under these conditions. This is further supported by our finding that BMSi values were comparable between patients with multiple fractures and those with a single fracture. In order, however, to better define the contribution of bone material properties to the susceptibility to high-energy trauma fractures, it is necessary to put this conclusion in context with the known important determinants of fracture risk, aBMD, and age. In agreement with some, but not all, reports [4–6], subjects with high-energy trauma fractures had significantly higher LS-, but not FN-aBMD, and more subjects had normal aBMD values compared to those with low-energy trauma fractures. In addition, and consistent with earlier reports [12, 21], we found no association between BMSi and aBMD values but between BMSi and age. These findings were also present in the group of high-energy trauma patients. Furthermore, BMSi values were similar in subjects with osteopenia and osteoporosis within each trauma group. Observational studies of large cohorts of women and men have shown that subjects with incident fractures had, as expected, lower aBMD values than non-fractured controls that were, however, not different between subjects with high- and those with low-energy trauma fractures [4–6]. Together with difficulties to quantify the force of trauma in clinical practice, these ob-

servations led to the recommendation to stop classifying fractures as high- and low-energy trauma and care for all patients with fractures in the same way and independently of the severity of the injury associated with the fracture [33]. Mackey et al. [6] showed that among men and women ≥ 65 years of two large prospective US cohorts (Study of Osteoporotic Fractures (SOF) and Osteoporotic Fractures in Men Study (MrOs)), the incidence of high-energy trauma nonspine fractures increased significantly in subjects with osteoporosis (aBMD T-scores ≤ −2.5 SD), and prevalence of osteoporosis was similar between subjects with high- and low-energy trauma fractures (36.2% vs 36.6% in women and 12.8% vs 14.8% in men). Therefore, subjects with osteoporosis were at high risk of fracture independently of the initiating trauma underscoring the importance of aBMD measurements in the assessment of all patients presenting with nonspine fractures. Although the prevalence of osteoporosis in our study needs to be interpreted with caution due to the small sample size, our findings are in line with this conclusion as 17.5% of subjects (predominantly men, approximately 20 years younger than those of the study of Mackey et al.) with high-energy trauma fractures had osteoporosis, a prevalence much higher than would have been expected in individuals of the same age in the general population [34]. Thus, a substantial number of relatively younger individuals (mean age 57 years) with high-energy trauma fractures was osteoporotic. In contrast, however, to the findings of the SOF and MrOs studies, the prevalence of osteoporosis in subjects with low-energy trauma fractures in our study was significantly higher (35%). Moreover, in the study of Mackey et al. in both men and women with osteopenia (T-scores between < −1 SD and > −2.5 SD), the adjusted incidence of traumatic fractures was not elevated compared to that of non-fractured subjects whereas the incidence of low-energy trauma fractures was. The incidence of traumatic fractures was further not related with age as was the case in subjects with low-energy trauma fractures. These observations combined raise questions about the effects of differences among subjects included in studies of different designs on clinical outcomes, but also of prevalent bone-related factors, other than aBMD, that affect bone fragility; such differences should be considered before generalizing reported results to all subjects with high-energy trauma fractures. A study conducted in trauma units in Austria reported the frequency of low- and high-energy trauma nonspine fractures over 13 years in more than 400,000 individuals of all ages [35]. The frequency of high-energy trauma fractures was much higher in the young and decreased with aging, while low-energy trauma fractures were much more frequent in older individuals. Importantly, the age at which an equal frequency of high- and low-energy trauma fractures was observed was 54 years in women and 70 years in men. In the absence of studies of fracture frequency following high-energy trauma, results of that study suggest that most observational studies tend to underestimate the frequency of high-energy trauma fractures particularly in younger individuals. Alternatively, differences in bone material properties may have contributed to observed differences

in frequency of high- and low-energy trauma fractures particularly in subjects without osteoporosis, who constitute by far the largest number of fractures [36]. These hypotheses need, however, to be tested in prospective studies. Related to these considerations is the important observation that a previous high-energy trauma fracture increases the risk of a new incident fracture in the same way as a low-energy trauma fracture does [5, 6, 37, 38]. Incident fractures after a high-energy trauma fracture represent, however, a minority of subsequent fractures (5.5% in the study of Leslie et al.). In addition, they occur mainly in older individuals, more likely to have osteoporosis, but not in individuals younger than 65 years in whom only a previous low-energy trauma fracture increased significantly the risk of subsequent fractures. Our study, the first to report IMI results in patients with fractures due to high-energy trauma, has strengths and limitations. Main strengths are the inclusion of treatment-naive subjects, the precise definition of the injury, and the a priori matching of the groups with high- and low-energy trauma fractures. Limitations include its cross-sectional design, the low number of participants that did not allow subgroup analyses, and the lack of a control group without fractures following a high-energy trauma. An additional limitation is a possible selection bias in the high- and low-energy trauma group. The former group might — due to the rapid and high loading event leading to such a fracture — more often be engaging in sporting activities and therefore reflect a more active population. A study by Sundh et al. suggested that exercise can increase BMSi [39]; thus, this group might have improved bone material properties compared to the general population. However, we did not include an activity questionnaire in the evaluation of subjects participating in the study, and this therefore can only be speculated.

In conclusion, our results demonstrate that bone material properties of patients with high-energy trauma fractures are higher than those of patients with low-energy trauma fractures and comparable with those previously reported in subjects without fractures. Further investigation in the measurement of BMSi in non-osteoporotic high-energy trauma fracture patients is warranted to determine whether IMI might help in identifying those with reduced bone strength.

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Chapter 4

Treatments of osteoporosis increase bone material strength index in patients with low bone mass

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Abstract

Summary Effects on bone material properties of two-year antiosteoporotic treatment were assessed using in vivo impact microindentation (IMI) in patients with low bone mineral density (BMD) values. Antiresorptive treatment, in contrast to vitamin D ± calcium treatment alone, induced BMD-independent increases in bone material strength index, measured by IMI, the magnitude of which depended on pretreatment values.

Introduction Bone material strength index (BMSi), measured by IMI in vivo, is reduced in patients with fragility fractures, but there is no information about changes in values during long-term therapy. In the present study, we assessed changes in BMSi in patients receiving antiosteoporotic treatments for periods longer than 12 months.

Methods We included treatment-naïve patients with low bone mass who had a BMSi measurement with OsteoProbe® at presentation and consented to a repeat measurement after treatment.

Results We studied 54 patients (34 women), median age 58 years, of whom 30 were treated with bisphosphonates or denosumab (treatment group) and 24 with vitamin D ± calcium alone (control group). There were no differences in clinical characteristics between the two groups with the exception of a higher number of previous fragility fractures in the treatment group. Baseline hip BMD and BMSi values were lower in the treatment group. After 23.1 ± 6.6 months, BMSi increased significantly in the treatment group (82.4 ± 4.3 vs 79.3 ± 4.1; $p < 0.001$), but did not change in the control group (81.5 ± 5.2 vs 82.2 ± 4.1; $p = 0.35$). Changes in BMSi with antiresorptives were inversely related with baseline values ($r = -0.43$; $p = 0.02$) but not with changes in BMD. Two patients in the control group with large decreases in BMSi values sustained incident fractures.

Conclusion In patients at increased fracture risk, antiresorptive treatments induced BMD-independent increases in BMSi values, the magnitude of which depended on pretreatment values.

Introduction

Impact microindentation (IMI), a method to assess tissue-level properties of cortical bone in vivo [1, 2], is being increasingly used in studies evaluating the contribution of such properties to bone fragility in humans [3–6]. The resistance of cortical bone to indentation, measured as bone material strength index (BMSi), was decreased in individuals with fragility fractures compared with appropriate controls in several studies [7–10]. It is not related to bone mineral density (BMD) values [7–10], but, as recently shown, BMSi most likely assesses subperiosteal bone material properties [11].

Short-term effects of antiosteoporotic treatments on BMSi have been examined in patients initiating glucocorticoid therapy [12]. Glucocorticoids induced rapid decreases of BMSi, while treatments prevented this decline or even increased BMSi values within 7 weeks, up to 20 weeks, with no concurrent changes in BMD values. These results strongly suggested that BMSi could detect early, treatment-induced changes in bone material properties in glucocorticoid-treated patients. In addition, increases in BMSi have been reported in postmenopausal women 3 months after a high impact exercise program [13], and in HIV-infected patients after 1 year treatment with antiretroviral agents [14] or following gastric bypass surgery in obese subjects with and without type 2 diabetes [15]. However, the effect of antiosteoporotic treatments on BMSi in patients at increased risk of fractures not receiving glucocorticoids has not been investigated. Moreover, osteoporosis is a chronic disease requiring long-term treatment, and it is currently unknown whether changes in BMSi over time might provide information, additional to those obtained by BMD measurements, in treated individuals.

In the present study, we addressed this question in individuals at increased risk of fractures by measuring BMSi with the handheld IMI device OsteoProbe® before and after treatments given for periods longer than 12 months.

Patients and methods

Study design

Observational study evaluating longitudinal changes in BMSi with antiresorptive agents or vitamin D ± calcium alone in women and men with low bone mass attending the outpatient clinic of the Center for Bone Quality of the Leiden University Medical Center (LUMC) between March 2015 and September 2018.

Patients

Included in the study were treatment-naïve subjects ≥ 18 years with osteopenia or osteoporosis who had an IMI measurement at presentation, were followed by one of the authors (NAD) for at least 1 year and consented to a repeat measurement. Exclusion criteria included any treatment affecting bone metabolism during follow-up—except calcium, vitamin D, bisphosphonates or denosumab—a metabolic bone disease other than osteoporosis, immobilization and local pathologies of the tibia or skin at the site of examination.

Treatment with antiresorptive agents (with vitamin D ± calcium) or vitamin D ± calcium alone was given according to Dutch national guidelines for the management of osteoporosis [16]. Treatment was initiated with oral bisphosphonates (BP) unless patients had epigastric complaints or did not want to receive oral BP; in these patients, iv BP or denosumab (DMAb) were given according to patients' preference and physician's judgement. All patients were followed at regular intervals in the outpatient clinic according to protocols of the Center for Bone Quality for the different treatment regimens. The study was approved by the Medical Ethical Committee of the LUMC, and written informed consent was obtained from all individuals included in the study.

Methods

Detailed medical history, clinical risk factors for fracture, fracture history with documentation of sites and dates of occurrence and information about use of medication were collected at baseline and follow-up visits. A fragility fracture was defined as any low-energy fracture, excluding those of the hands, feet and skull. Reasons and time of discontinuation or change to another antiresorptive agent during follow-up were recorded.

Laboratory measurements

Serum calcium (albumin-corrected), creatinine and alkaline phosphatase were measured by semiautomated techniques. Plasma intact PTH was measured by immulite 2500 (Siemens Diagnostics, Breda, The Netherlands) and serum 25-hydroxyvitamin D (25-OH D) by the 25-OH-vitamin D TOTAL assay (DiaSorin D.A./N.V., Brussels, Belgium).

Bone mineral density

Areal BMD was measured at the lumbar spine (L1–L4) and the left and right hip by dual-energy X-ray absorptiometry (DXA) with the Hologic QDR 4500 (Hologic Inc., Waltham, MA, USA). Average BMD values of the hip were used in the analysis. NHANES III reference values compatible with reference values of the Dutch popula-

tion were used to calculate T-scores. Osteopenia and osteoporosis were diagnosed according to WHO criteria.

Vertebral fracture assessment

Spine radiographs for the detection of vertebral deformities were performed at baseline in all patients. During follow-up, radiographs were only performed if there was loss of height > 3 cm or clinical complaints. Radiographs were independently evaluated by two of the authors (NAD and MS) using the semiquantitative method of Genant et al. [17], and only fractures grade 2 or higher were considered in the analysis.

Bone material strength index

BMSi was measured in all patients by IMI using the handheld microindenter device (OsteoProbe® RUO, Active Life Scientific, CA, USA) at the midshaft of the tibia. Measurements were performed by two experienced operators (NAD and FM) according to our previously published protocol [7, 8, 18, 19]. The patient was placed in a decubitus supine position with the tibia in external rotation. The measurement site was defined as the mean distance between the medial malleolus and the distal apex of the patella. After disinfection and local anaesthesia of the skin and periosteum with lidocaine 1%, the test probe was gently inserted in the skin until the bone surface was reached. It was ensured that the test probe was always perpendicular to the bone surface during measurements. The operator was not allowed to check the measurements on the computer screen before these were classified as "well performed", "adequate" or "poorly performed". Measurements were classified as "well performed" when the test probe was exactly perpendicular to the bone surface, as "adequate" when the test probe was within acceptable deviation from the bone surface [2] and as "poorly performed" when the operator judged that the test probe was not appropriately placed. "Poorly performed" measurements are usually due to slipping of the test probe, moving of the subject's leg, indenting the same spot twice, or failure to place the device perpendicularly to the bone surface and were manually discarded. In addition, the first measurement was systematically discarded since there is often inadequate penetration of the probe through the periosteum. After at least five adequate measurements (range 6 to 15, mean 10.0 ± 1.8 measurements), five additional measurements were performed on a polymethylmethacrylate (PMMA) calibration phantom. BMSi was calculated by the computer software. The operator was not blinded to the patient's treatment, but was blinded to baseline BMSi results, and classification of measurements was done before checking the results on the computer screen. The intraobserver coefficient of variation (CV) was 2.2%, and the interobserver CV was 1.6%.

Statistical analysis

Results are presented as mean ± SD unless otherwise stated. Descriptive statistics were used to describe clinical and laboratory parameters. Between-group differences in baseline characteristics were assessed using a Student's t test or Mann-Whitney U test and chi-square test or Fisher's exact test for normally and not normally distributed continuous and for categorical variables, respectively. Within-group changes in BMSi and BMD were assessed using a paired t test. Normality of the distribution of BMSi and BMD was checked by a Kolmogorov-Smirnov test and visually with histograms. Between-group differences in % changes from baseline to follow-up measurements were assessed by a Student's t test. ANCOVA models with % change in BMSi as outcome variable, adjusted for baseline BMSi, BMD and fragility fracture, were used to compare % change in BMSi between groups. Correlations between BMSi and BMD values and between % changes in BMSi and % changes in BMD or duration of treatment were examined by a Pearson's test. To determine independent effects of factors possibly influencing changes in BMSi, a multiple linear regression analysis was used. Age, gender, fragility fracture, baseline BMSi, treatment duration as well as examiner of the first and the second IMI measurement, respectively, were included in the model. A power calculation was performed using a difference of 3.1% in BMSi values. This difference was previously found between patients with and patients without fragility fractures (BMSi 79.9 ± 0.6 vs 82.4 ± 1.0) and considered to be clinically relevant [7]. The sample size to detect this difference in BMSi from baseline to follow-up with a standard deviation of 5 and power of 0.8 at a significance level of 0.05 was calculated to be 24 per group. All analyses were performed using SPSS software for Windows (version 25.0; SPSS Inc., Chicago, IL, USA). A p value < 0.05 was considered to be statistically significant. Graphs were constructed with Graphpad Prism (version 8.0; Graphpad Software Inc., La Jolla, CA, USA).

Results

Fifty-four patients were included in the study (Fig. 1). These were 34 women and 20 men with median age 58.0 years (IQR 48.5–63.3 years). Thirty-one patients had osteoporosis (57.4%) and 23 had osteopenia (42.6%); 23 patients (7 men) had sustained one or more fragility fractures (11 vertebral, 2 hip, 14 non-hip/non-vertebral) at the time of the first IMI measurement (Table 1). Mean time to second measurement was 23.1 ± 6.6 months. Baseline characteristics of patients who were excluded from the study, either because they were referred to their general practitioners, were followed by other physicians in our center or were lost to follow-up (Fig. 1), were not different from those of the included patients; age 56.2 ± 14.2 vs 55.3 ± 13.0 years, p = 0.637; female gender 59.5% vs 63.0%, p = 0.665; prevalent fragility fractures 47.7%

vs 42.6%, p = 0.500; BMSi 80.7 ± 5.6 vs 80.6 ± 4.3, p = 0.894. Of the 54 patients included in the analysis, 30 started antiresorptive therapy after a median of 4 days following the first IMI measurement (treatment group); 23 patients received BPs (16 oral, 7 iv) and 7 DMAb. Twenty-four patients received vitamin D ± calcium alone (control group) (Table 1). Patients in the treatment group had sustained significantly more fragility fractures compared with those of the control group (25 vs 4, p < 0.01), due mainly to the higher prevalence of vertebral fractures in the former group (14 vs 0, p < 0.01).

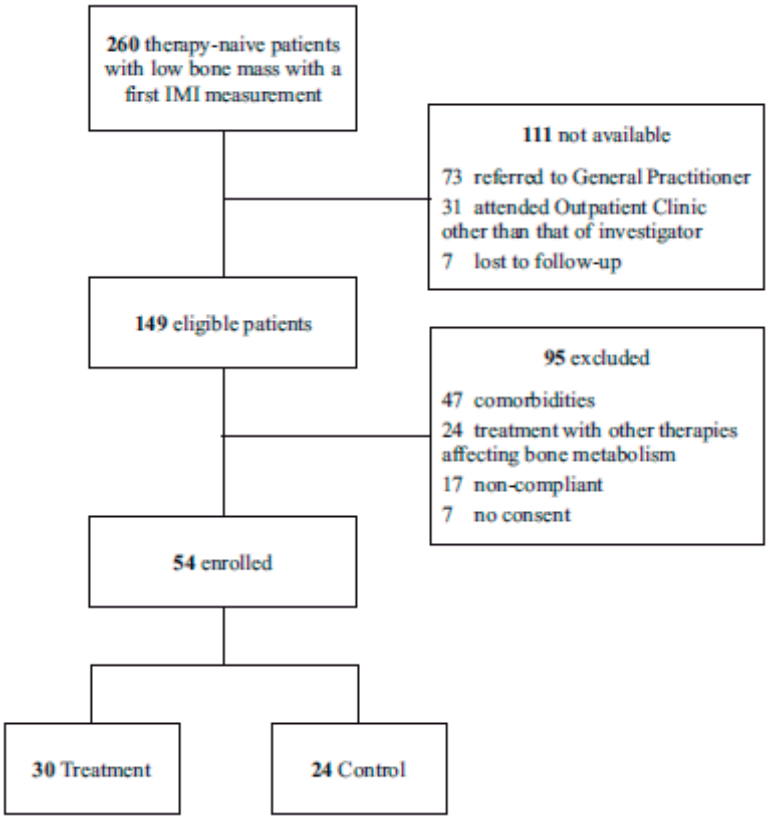

Fig. 1 Patient flowchart

Table 1 Baseline characteristics of 54 patients

Characteristics	All patients	Treatment	Control	p value
n	54	30	24	
Age, years	58.0 ± 1.8	59.0 ± 1.8	55.0 ± 3.2	0.16
Male/female	20/34	8/22	12/12	0.11
BMI, kg/m2	24.2 ± 3.6	24.0 ± 3.4	24.5 ± 4.0	0.67
Smoking, *n*	7	6	1	0.12
Alcohol use ≥ 3U/d, *n*	5	2	3	0.65
Previous fragility fracture, *n* (%)	23 (42.6)	19 (63.3)	4 (16.7)	0.001
Hip fracture, *n* (%)	2 (3.7)	2 (6.7)	0 (0.0)	0.50
NHNV fracture, *n* (%)	14 (25.9)	10 (33.3)	4 (16.7)	0.17
Vertebral fracture, *n* (%)	11 (20.4)	11 (40.0)	0 (0.0)	0.001
Calcium[a], mmol/L	2.32 ± 0.08	2.34 ± 0.08	2.31 ± 0.08	0.11
Creatinine[b], umol/L	76.1 ± 15.0	74.0 ± 13.8	78.8 ± 16.2	0.24
25-OH D[c], nmol/L	80.9 ± 27.7	78.7 ± 29.0	83.6 ± 26.4	0.52
PTH[d], pmol/L	3.5 ± 1.3	3.6 ± 1.6	3.2 ± 0.9	0.13
LS-BMD T-score	− 2.1 ± 0.8	− 2.3 ± 0.7	− 1.9 ± 0.9	0.10
FN-BMD T-score	− 1.7 ± 0.7	− 1.9 ± 0.7	− 1.5 ± 0.6	0.08
TH-BMD T-score	− 1.3 ± 0.7	− 1.5 ± 0.6	− 1.1 ± 0.8	0.026
BMSi	80.6 ± 4.3	79.3 ± 4.1	82.2 ± 4.1	0.014

NHNV, Non-hip/non-vertebral; BMD, bone mineral density; LS, lumbar spine; FN, femoral neck; TH, total hip; BMSi, bone material strength index. Values are expressed as mean ± SD. Age is expressed as median ± SEM. p values are displayed for treatment vs control

[a] Calcium (albumin-corrected) reference range, 2.15–2.55 mmol/L

[b] Creatinine reference range, 64–104 umol/L for males; 49–90 umol/L for females

[c] 25-OH vitamin D reference range, 50–250 nmol/L

[d] PTH reference range, 0.7–8.0 pmol/L

Bone mineral density

In the treatment group, 20 patients had osteoporosis (T-score ≤ −2.5) and 10 had osteopenia (T-score between −2.5 and −1.0); in the control group, 11 patients had osteoporosis and 13 had osteopenia (p = 0.124 between the two groups). Lumbar spine (LS) BMD was lower in the treatment group, but the difference between the two groups was not significant (0.81 ± 0.08 vs 0.86 ± 0.10 g/cm2, p = 0.06); femoral neck (FN) BMD and total hip (TH) BMD were significantly lower in the treatment group (FN 0.66 ± 0.09 vs 0.70 ± 0.09 g/cm2, p = 0.041; TH 0.77 ± 0.10 vs 0.84 ± 0.12 g/cm2, p = 0.016). Antiresorptive treatment was associated with significant increases in LS-BMD (6.3 ± 5.3%) and TH-BMD (1.1 ± 2.9%) but not in FN-BMD (1.2 ± 3.6%) (Fig. 2). In the control group, LS-BMD did not change significantly during follow-up (−1.3 ± 3.6%,), while hip BMD decreased significantly at both measured sites (FN-BMD −2.7 ± 4.6%, TH-BMD −2.4 ± 2.9%) (Fig. 2).

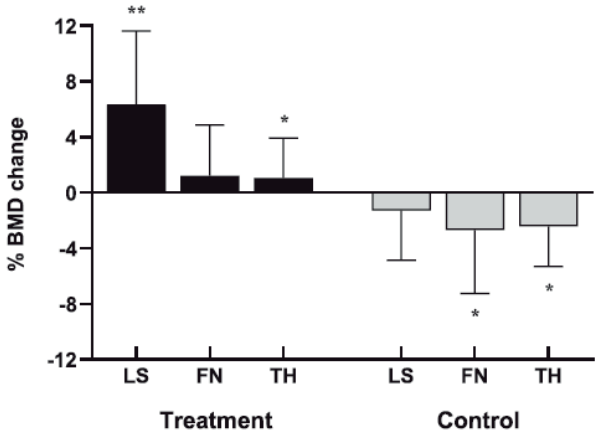

Fig. 2 Mean (±SD) percentage changes in bone mineral density (BMD) at follow-up compared with baseline in the treatment (black bars) and control group (grey bars) at the lumbar spine (LS), femoral neck (FN) and total hip (TH). *Significantly different from baseline, $p < 0.05$ and **$p < 0.001$

Bone material strength index

Consistent with our previous studies, baseline BMSi values of all studied patients were not associated with baseline BMD values at any site (LS r = −0.072, p = 0.617; FN r = 0.067, p = 0.631; TH r = 0.048, p = 0.728) and were significantly lower in patients with fragility fractures compared with those without (78.5 ± 3.0 vs 82.1 ± 4.5, p = 0.002).

Changes during follow-up

Baseline BMSi values of the treatment group were significantly lower than those of the control group (Table 1) and increased significantly by 4.0 ± 5.2% to values similar to those of the control group at baseline (from 79.3 ± 4.1 to 82.4 ± 4.3, $p < 0.001$) (Fig. 3). The increase in BMSi with antiresorptive treatment was inversely related with baseline BMSi (r = −0.432, p = 0.017) (Fig. 4) but not with the duration of treatment (r = 0.089 p = 0.639) or with the BMD changes (LS r = −0.076, p = 0.712; FN r = 0.266, p = 0.155; TH r = −0.031, p = 0.870). In contrast, in the control group, BMSi values decreased during the period of observation but not significantly (from 82.2 ± 4.1 to 81.5 ± 5.2, p = 0.353; −0.7 ± 3.9%) (Fig. 3). The difference in % BMSi changes between the two groups was significant, $p < 0.001$ (also after adjusting for baseline BMSi (p = 0.004), baseline BMD (p = 0.007) and prevalent fragility fractures (p = 0.016)). In both BP- and DMAb-treated patients for the whole observation period, BMSi increased significantly (BP from 80.1 ± 4.6 to 82.5 ± 4.4 (3.1 ± 5.6%), p = 0.037; DMAb from 78.1 ± 3.1 to 84.2 ± 4.3 (7.8 ± 3.1%), p = 0.001). The percentage increase in BMSi was higher

in the DMAb-treated patients (p = 0.014), but after adjusting for baseline BMSi, the difference between the two treatments was not significant anymore (7.1 ± 4.8% vs 3.4 ± 4.7%, p = 0.104). Numbers however were small.

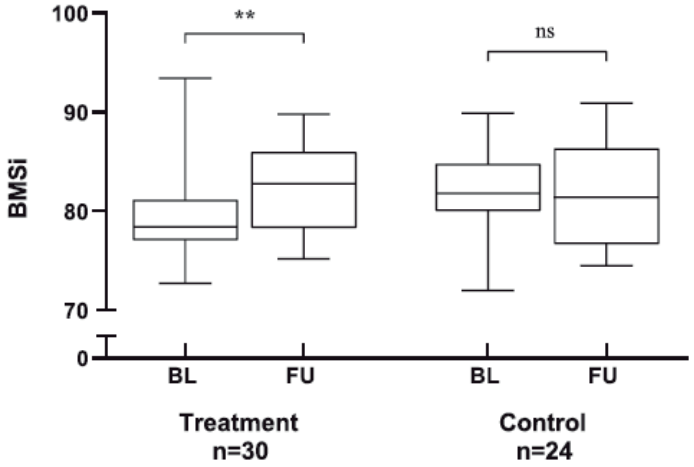

Fig. 3 Bone material strength index (BMSi) in patients with antiresorptive treatment (treatment) and those without antiresorptive treatment (control) at baseline (BL) and follow-up (FU). Data are shown in boxwhisker plots and statistical differences are displayed for BMSi. Boxes indicate median and interquartile range. Bars indicate minimum and maximum values. **p < 0.001

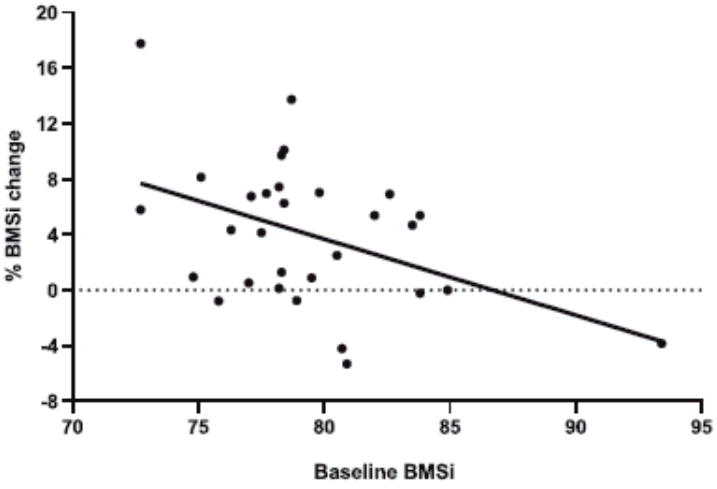

Fig. 4 Relationship between bone material strength index (BMSi) at baseline and % BMSi change from baseline to follow-up in 30 patients treated with antiresorptive agents. r = −0.432, p = 0.017. Absence of change in BMSi is indicated by the dashed line

Fractures

During the period of observation, there were no fragility fractures in the treatment group while two patients of the control group sustained fragility fractures. In the first patient, a decline of BMSi by 8.5% was documented 17 months before a humerus fracture; the corresponding decreases of LS-, FN- and TH-BMD were −2.2%, −1.3% and −2.7% to T-scores of −1.5, −0.9 and −0.4, respectively. New assessment revealed no causes of secondary osteoporosis. The second patient sustained a Weber B ankle fracture 18 months after the second measurement that showed an 8.9% decline of BMSi; the corresponding decreases of LS-, FN- and TH-BMD were −4.8%, −7.0% and −4.3% to T-scores of −2.2, −1.7 and −0.4, respectively.

Discussion

This study demonstrates that treatment with BPs and DMAb, given for a mean period of 2 years, increases BMSi values, measured by impact microindentation, in patients with low bone mass and increased risk of fracture. The values of BMSi reached with treatment were not different from baseline values of the patients of the control group as well as from those previously reported in patients with low bone mass without fractures [7, 8]. The magnitude of these changes was, however, different among treated patients and depended on baseline values with larger increases observed in the patients with lower baseline values. Our results are, therefore, in agreement with those obtained after short-term treatment of patients receiving glucocorticoids [12]. The pattern of BMSi changes by antiresorptive therapy was further similar to that observed after treatment of HIV-infected patients with antiretroviral agents or following gastric bypass surgery in obese subjects with and without type 2 diabetes [14, 15].

The increases in BMSi values observed in our and other studies [12, 14, 15] were not related with changes in BMD illustrating, in a different setting, the independence of measurements of bone material properties from those of mineral density as also reported previously in cross-sectional studies [7–10, 20–28]. We recently confirmed the lack of a relationship between BMSi and BMD by direct measurements of bone mineral density distribution (BMDD) by quantitative backscattered electron imaging (qBEI) in whole bone tissue in iliac crest bone biopsies obtained concurrently with BMSi measurements in patients with a wide range of BMD [11].

In the present study, patients treated with antiresorptive agents had lower baseline BMSi values and a higher number of fragility fractures than those treated with vitamin D ± calcium alone. Notably, BMSi was not considered in treatment decision

that was based on conventional tools to assess fracture risk. Guidelines for use of BMSi measurements by OsteoProbe® in clinical practice are not yet available, and their value is investigated in research settings, as in our study. Results, however, strengthen the notion that measurements of BMSi may be a useful addition to currently available tools for the assessment of fracture risk.

The results of our study and that of Mellibovsky et al. in glucocorticoid-treated patients [12] demonstrate that BMSi, measured by IMI, increases by treatments of osteoporosis. We show, in addition, that these increases are not related to treatment duration and appear to have a plateau which is essentially not different from normal values and might represent the maximum value that can be achieved with treatment. This plateau was reached with both DMAb and BP, and the significantly higher increases in BMSi values with DMAb are likely due to the lower baseline BMSi values of patients treated with this more potent agent, but numbers were small. The mechanism responsible for the increase in BMSi by antiresorptives has not yet been established. We recently showed that BMSi measured by OsteoProbe® is significantly associated with bone material properties, measured by Raman spectroscopy, of subperiosteal bone in patients with different skeletal disorders and a wide range of BMD, with or without fractures [11]. We have now extended this study, and we measured material properties of the whole cortex of these biopsies by Fourier transform infrared imaging (FTIRI), a spectroscopic method more suitable for the measurement of compositional bone changes in large areas (manuscript in preparation). In this analysis, BMSi values were strongly associated, $p < 0.01$, with the mineral to matrix ratio (MM), the most widely measured bone material property, that, differently from other measurements of mineral density, directly measures and accounts for the amount of organic matrix in the volume analysed [29]. In rodents, MM correlates with ash weight and is directly proportional to bending stiffness and failure moment being superior to total mineral density, measured by micro-CT, in predicting bone strength [30, 31]. Bisphosphonates and hormone replacement therapy given for 1 to 3 years to animals or humans increased significantly spectroscopically measured MM [32–35]. Moreover, inhibition of receptor activator of nuclear factor kappa-B ligand (RANKL) by osteoprotegerin (OPG) significantly increased MM in genetically modified ovariectomized mice to levels of wild-type animals (E. Paschalis personal communication). It may, therefore, be that BMSi measurements capture the observed increases in MM by antiresorptive therapies. Notably, prolongation of treatment with bisphosphonate, as in the FLEX study with alendronate, is not associated with continuous increase in MM [36] indicating that the amount of mineral taken up by the matrix in non-pathological mineralization is limited, or self-regulated; this finding is similar to the observed plateau of bone mineralization between 5 and 10 years treatment of patients with osteoporosis with denosumab [37] and may explain

the lack of further increases in BMSi values when these reach normal levels shown in our study.

Notably, patients on antiresorptive treatments in our study sustained no fractures, whereas fractures occurred only in two patients in the control group. These two patients showed significant decreases in BMSi values (−8.5% and −8.9%) during follow-up, whereas the corresponding decreases in BMD were variable and ranged between −1.3% and −7.0%. Moreover, in a cross-sectional study of postmenopausal women on long-term treatment with bisphosphonates (4 to 14 years) BMSi values were significantly lower in women with incident fractures compared with those without fractures [28]. Thus, IMI measurements may not only be a useful, complementary investigation in the assessment of fracture risk but may also provide additional long-term information about the effects of treatments on bone material properties and their association to bone fragility. This needs, however, to be confirmed in appropriately designed studies. A limitation of the study is the inclusion of only patients followed by one of the authors. However, baseline characteristics of patients who had a baseline measurement but were not included in the study did not differ from those of patients reported here and treatment protocols were the same. Moreover, all measurements were performed by two experienced investigators that minimized intra- and interobserver variability, a known limitation of the application of IMI technique [1, 2, 6].

In summary, our study, the first to investigate longitudinal changes in BMSi with antiosteoporotic treatments in patients with low bone mass and increased fracture risk, demonstrates that IMI can capture BMD-independent, treatment-induced increases in BMSi, the magnitude of which depends on pretreatment values.

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Chapter 5

Bone Material Strength Index as Measured by Impact Microindentation is Low in Patients with Primary Hyperparathyroidism

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Abstract

Context: In primary hyperparathyroidism (PHPT) bone mineral density (BMD) is typically decreased in cortical bone and relatively preserved in trabecular bone. An increased fracture rate is observed however not only at peripheral sites but also at the spine, and fractures occur at higher BMD values than expected. We hypothesized that components of bone quality other than BMD are affected in PHPT as well.

Objective: To evaluate bone material properties using impact microindentation (IMI) in PHPT patients.

Methods: In this cross-sectional study, the Bone Material Strength index (BMSi) was measured by IMI at the midshaft of the tibia in 37 patients with PHPT (28 women), 11 of whom had prevalent fragility fractures, and 37 euparathyroid controls (28 women) matched for age, gender, and fragility fracture status.

Results: Mean age of PHPT patients and controls was 61.8 ± 13.3 and 61.0 ± 11.8 years, respectively, $P = .77$. Calcium and PTH levels were significantly higher in PHPT patients but BMD at the lumbar spine (0.92 ± 0.15 vs 0.89 ± 0.11, $P = .37$) and the femoral neck (0.70 ± 0.11 vs 0.67 ± 0.07, $P = .15$) were comparable between groups. BMSi however was significantly lower in PHPT patients than in controls (78.2 ± 5.7 vs 82.8 ± 4.5, $P < .001$). In addition, BMSi was significantly lower in 11 PHPT patients with fragility fractures than in the 26 PHPT patients without fragility fractures (74.7 ± 6.0 vs 79.6 ± 5.0, $P = .015$).

Conclusion: Our data indicate that bone material properties are altered in PHPT patients and most affected in those with prevalent fractures. IMI might be a valuable additional tool in the evaluation of bone fragility in patients with PHPT.

Introduction

Primary hyperparathyroidism (PHPT) is a common endocrine disorder characterized by an inappropriate excess of parathyroid hormone (PTH) resulting in elevated serum calcium, usually caused by an adenoma of the parathyroid glands. PTH plays an important role in the maintenance of bone mass and integrity, but its continuous excess in PHPT increases bone turnover in favor of bone resorption, resulting in bone loss predominantly at cortical sites with relative preservation at trabecular sites (1-4). An increased fracture rate, however, has been found at all relevant skeletal sites including the spine, and fractures occur at higher bone mineral density (BMD) levels than expected (3). These findings suggest that in PHPT factors contributing to bone quality other than BMD are also affected. These factors include bone architecture on the macro- and microlevels, and tissue material properties. Microarchitecture of cortical and trabecular bone at the radius and the tibia have been assessed in postmenopausal women with PHPT using high-resolution peripheral quantitative computed tomography and found to be altered compared with healthy controls (5). Tissue material properties of bone, however, are the least understood and the hardest to evaluate and could until recently only be assessed ex vivo by using transiliac bone biopsy specimens. Since the introduction of the impact microindentation (IMI) technique, these properties have become measurable in humans in vivo at the midshaft of the tibia (6). The resistance of cortical bone to indentation, measured as Bone Material Strength index (BMSi), is altered in patients with fragility fractures compared with patients without fractures, independently of BMD (7-10), and is strongly associated with material properties of subperiostal mineralized bone surface (11). In the present study, we aimed to evaluate if bone material properties, as assessed by IMI, are altered in patients with primary hyperparathyroidism compared with euparathyroid controls. In addition, we aimed to evaluate whether BMSi differed between PHPT patients with or without fragility fractures.

Patients and Methods

Study Design

This was a cross-sectional study evaluating BMSi in men and women with PHPT and in euparathyroid controls with osteoporosis, osteopenia, or normal BMD. Patients and controls were studied at the outpatient clinics of Leiden University Medical Center (LUMC), a national expertise center for PHPT. The study was approved by the Medical Ethics Committee of the LUMC and written informed consent was obtained from all individual participants included in the study. All procedures performed were in accordance with the ethical standards of the institutional research committee

and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Patients with PHPT

Patients aged 18 years or older with a diagnosis of PHPT attending the outpatient clinic of the Department of Endocrinology of LUMC between October 2018 and February 2020 were asked to participate in the study. The diagnosis of PHPT was made in the case of a repeatedly elevated calcium concentration (albumin corrected), with nonsuppressed PTH in the absence of vitamin D deficiency.

Controls

Men and women with or without fragility fractures who had normal serum calcium levels and no history of hyperparathyroidism served as controls. They were recruited from the outpatient clinics of the Center for Bone Quality or from the regional Fracture Liaison Service of LUMC and were matched for age and gender and the presence or absence of fragility fractures. Exclusion criteria for both groups were a metabolic bone disease other than osteoporosis, any untreated endocrine disorder, severe liver insufficiency, or chronic kidney disease stage IV or V, immobilization, a contraindication for IMI measurement (12), or inability to provide informed consent. In addition, the use of any treatment affecting bone metabolism other than calcium and vitamin D was also an exclusion criterion with the exception of bisphosphonate (BP) or denosumab (DMAb) in patients with PHPT.

A full medical history, use of medication including agents affecting bone metabolism, vitamin D supplementation, dietary calcium intake, and a detailed fracture history with documentation of sites and dates of occurrence of fractures were obtained from all subjects. A fragility fracture was defined as any low-energy fracture, excluding those of the hands, feet, and skull. Data on clinical risk factors for fracture as used in the FRAX algorithm were obtained from all subjects (FRAX) (13).

Laboratory Parameters

Serum calcium (albumin corrected), phosphate, creatinine, and alkaline phosphatase concentrations were measured using semi-automated techniques. Plasma intact PTH was measured by Immulite 2500 (Siemens Diagnostics, Breda, The Netherlands) and serum 25-hydroxyvitamin D concentrations by the 25-OH-vitamin D TOTAL assay (DiaSorin D.A./N.V., Brussels, Belgium).

Bone Mineral Density

Areal bone mineral density (BMD) was measured at the lumbar vertebrae (L1-L4) and at the left and right hip using dual-energy x-ray absorptiometry (DXA) with the Hologic QDR Discovery A (Hologic, Bedford, MA, USA). Mean BMD values of the

femoral neck were used in the analysis. NHANES III reference values compatible with reference values of the Dutch population were used to calculate T-scores. Osteopenia and osteoporosis were diagnosed according to World Health Organization criteria.

Vertebral Fracture Screening

Single energy x-ray lateral vertebral fracture assessment (VFA) images of the spine (T4-L4) by DXA or conventional anteroposterior and lateral radiographs of the thoracic and lumbar spine were performed following standard protocols for the detection of vertebral deformities at time of inclusion. Images were independently evaluated by 2 experienced readers using the semiquantitative method of Genant (14).

Impact Microindentation

BMSi was measured in all patients by IMI using a handheld microindenter device (OsteoProbe® RUO, Active Life Scientific, CA, USA) on the midshaft of the tibia as described previously (15). In brief, the patient was placed in a decubitus supine position with the tibia in external rotation to orient the flat surface of the medial tibia diaphysis in a horizontal position. The measurement site was defined as the mean distance between the medial malleolus and the distal apex of the patella. Following disinfection of the area and local anesthesia of the skin and periosteum with lidocaine 1%, the test probe was gently inserted in the skin until the bone surface was reached. After at least 5 adequate measurements, 5 additional measurements were performed on a polymethylmethacrylate calibration phantom. BMSi was calculated by the computer software. The intra-observer and interobserver coefficient of variation in our center are 2.2% and 1.6%, respectively.

Statistical Analysis

Results are presented as mean ± SD unless stated otherwise. Descriptive statistics were used to describe clinical and laboratory parameters. Normality assumptions were checked by inspection of histograms and tested by a Kolmogorov–Smirnov test. Differences in baseline characteristics between patients and controls were assessed using a paired t-test or Wilcoxon signed ranks test and McNemar's test for normally and not normally distributed continuous and for categorical variables, respectively. Conditional logistic regression was used to assess BMSi and BMD values adjusted for body mass index (BMI) to compare BMSi and BMD values between patients and controls. Correlations between BMSi values and patients' or disease characteristics were examined by Pearson's and Spearman's tests for normally and not normally distributed variables, respectively. Based on pilot data from our center, the sample size to detect a significant difference in BMSi between groups with a power of 0.8 at a significance level of .05 is 33. $P < .05$ was considered to be

statistically significant. All analyses were performed using SPSS software for Windows (version 25.0; SPSS Inc., Chicago, IL, USA) and graphs were constructed with Graphpad Prism (version 8.0; Graphpad Software Inc., La Jolla, CA, USA).

Results

Thirty-seven of 41 eligible patients with PHPT agreed to take part and were included in the study (Fig. 1). Median time since diagnosis was 8.0 months (interquartile range [IQR] 2.0-54.5 months) and mean age at diagnosis was 59.3 ± 14.1 years. At time of inclusion, 12 patients (32.4%) were using antiresorptives (n = 9; 4 oral BP, 4 iv BP, 1 DMAb) for a median duration of 29 months (IQR 9.5-61.0 months) and/or cinacalcet (n = 7), a calcimimetic drug. Reasons for treatment with antiresorptives in 9 of these 12 patients were severe hypercalcemia in 4 patients and a DXA diagnosis of osteoporosis in a patient with a contraindication for surgery. In 4 patients the antiresorptive treatment had been initiated due to osteoporosis or osteopenia with and without fractures before the diagnosis of PHPT.

Patients' characteristics and laboratory measurements of the 37 patients with PHPT (28 women) and 37 matched euparathyroid controls (28 women) are shown in Table 1. By design, the 2 groups were comparable in age and gender, and also comparable regarding the number of subjects with prevalent fragility fractures, 11 (29.7%) in PHPT patients and 11 (29.7%) in controls (P = 1.00). BMI was significantly higher in PHPT patients, who also—by definition—had higher serum calcium and PTH levels than controls (Table 1). Lumbar spine (LS) and femoral neck (FN) BMD were comparable between the 2 groups (Fig. 2A), also after adjusting for BMI (LS P = .871, FN P = .488).

Bone Material Strength index

BMSi was significantly lower in patients with PHPT than in controls, 78.2 ± 5.7 versus 82.8 ± 4.5, P < .001 (Fig. 2B), also after adjusting for BMI (P = .001), and when excluding the 9 patients who were using antiresorptives (P < .001).

In PHPT patients, BMSi did not differ between men and women (79.6 ± 4.4 versus 77.7 ± 6.1, P = .404), and there was no correlation between BMSi and age (r = -0.131, P = .440) or BMI (r = −0.074, P = .663).

In contrast, there was a significant relationship between BMSi and time since diagnosis (r = −0.382, P = .020), with lower BMSi the longer the disease duration. There was also a significant negative relationship between BMSi and plasma PTH levels (r = −0.358, P = .030), and between BMSi and serum calcium concentrations (r = −0.406, P = .013).

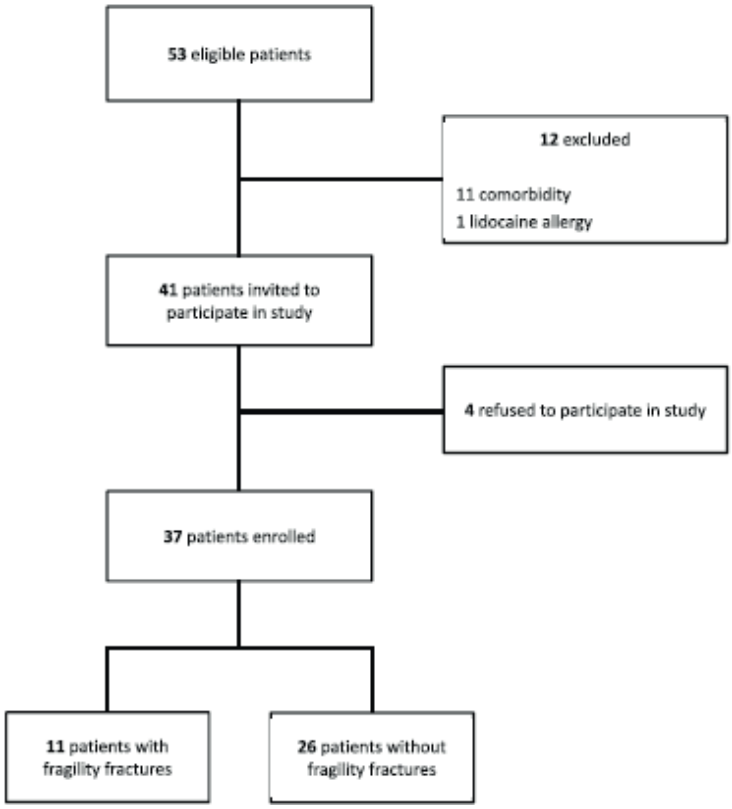

Figure 1. Patient flowchart.

Table 1. Characteristics of patients with primary hyperparathyroidism and controls

	PHPT (n = 37)	Controls (n = 37)	P value
Age, years	61.8 ± 13.3 (range 37-84)	61.0 ± 11.8 (range 40-83)	.768
Male/female	9/28	9/28	1.00
BMI, kg/m2	27.2 ± 0.9	24.7 ± 0.5	**.031**
Smoking, n (%)	0 (0.0)*	0 (0.0)*	NA
Alcohol use ≥3 U/d, n (%)	2 (5.4)*	2 (5.4)*	1.00
Time since diagnosis, months	8 (IQR 2-55)	NA	NA
Urolithiasis, n (%)	6 (16.2)	NA	NA
Fragility fracture any, n (%)	11 (29.7)	11 (29.7)	1.00
History of hip fracture, n (%)	1 (2.7)	2 (5.4)	1.00
Prevalent vertebral fracture (radiological), n (%)	5 (13.5)	5 (13.5)	1.00
History of NHNV fracture, n (%)	7 (18.9)	8 (21.6)	.772
Calcium[a], mmol/L	2.7 ± 0.2	2.3 ± 0.1	**<.001**
Phosphate[b], mmol/L	0.9 ± 0.2	1.1 ± 0.1	**<.001**
PTH[c], pmol/L	10.2 ± 1.6	3.3 ± 0.2	**<.001**
Creatinine[d], µmol/L	72.0 ± 3.2	72.0 ± 3.3	.559
25-OH Vit D[e], nmol/L	78.1 ± 27.5	79.4 ± 31.1	.847
LS BMD, g/cm2	0.92 ± 0.15	0.88 ± 0.11	.329
T-score LS	−1.1 ± 1.3	−1.6 ± 1.1	.154
FN BMD, g/cm2	0.70 ± 0.11	0.67 ± 0.07	.148
T-score FN	−1.5 ± 1.0	−1.7 ± 0.7	.298
BMSi	78.2 ± 5.7	82.8 ± 4.5	**<.001**

Values are expressed as mean ± SD. BMI, PTH, and creatinine are expressed as median ± SEM. *P* values in bold are significant. Abbreviations: BMD, bone mineral density; BMI, body mass index; BMSi, Bone Material Strength index; FN, femoral neck; LS, lumbar spine; NHNV, nonhip nonvertebral; PTH, parathyroid hormone. *Information about smoking status and alcohol consumption was missing in 1 patient and in 1 control. [a]Calcium (albumin-corrected) reference range, 2.15-2.55 mmol/L. [b]Phosphate reference range, 0.9-1.5 mmol/L. [c]PTH reference range, 0.7-8.0 pmol/L. [d]Creatinine reference range, 64-104 µmol/L for males; 49-90 µmol/L for females. [e]25-OH Vit D reference range, 50-250 nmol/L.

There was no relationship between BMSi and BMD neither at the LS (r = −0.222, *P* = .194) nor at the FN (r = −0.041, *P* = .813). Although BMSi tended to be higher in the 9 patients treated with antiresorptives than in the 28 patients without (80.7 ± 7.4 versus 77.4 ± 5.0, *P* = .133), this was not statistically significant. All findings of this study did not change when excluding the 12 patients using antiresorptives and/or cinacalcet (Supplemental Tables 1 and 2 (16)).

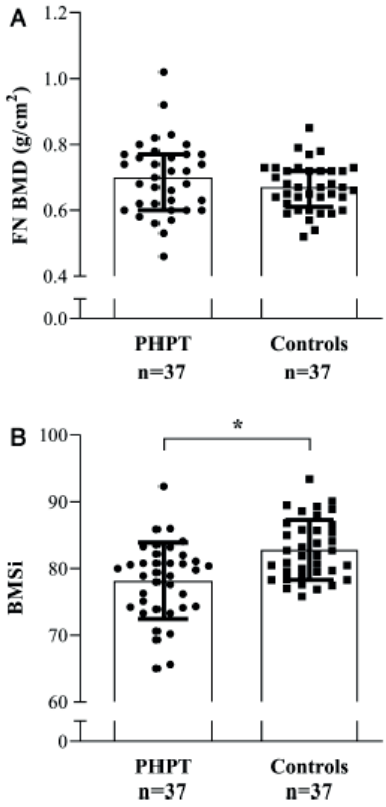

Figure 2. (A) Mean femoral neck bone mineral density (FN BMD) and (B) Bone Material Strength index (BMSi) in patients with PHPT and controls. Data are shown in box-whisker plots and statistical differences are displayed for BMSi. Boxes indicate median and interquantile range. Bars indicate minimum and maximum values. ***P*** < .001.

BMSi and Fractures

Baseline characteristics and laboratory measurements of PHPT patients with and without fragility fractures are shown in Table 2. BMSi was significantly lower in the 11 PHPT patients with prevalent fragility fractures than in the 26 PHPT patients without, BMSi 74.7 ± 6.0 versus 79.6 ± 5.0, *P* = .015 (Fig. 3), also after adjusting for BMD (*P* = .017). Patients with fractures tended to use cinacalcet more frequently and also appeared to have a lower FN BMD (Table 2). A DXA diagnosis of osteoporosis was established in 6 of them, while 4 had osteopenia and 1 normal BMD. BMSi of the 11 PHPT patients with fragility fractures was also significantly lower than the BMSi of the 11 controls with fragility fractures, 74.7 ± 6.0 versus 80.3 ± 4.9 (*P* = .010), while BMD was comparable between the 2 groups at both the LS (0.86 ± 0.16 versus 0.91 ± 0.12, *P* = .369) and the FN (0.62 ± 0.08 versus 0.66 ± 0.08, *P* = .329).

Table 2. Characteristics of patients with primary hyperparathyroidism with and without fragility fractures

	Fracture (n = 11)	No fracture (n = 26)	P value
Age, years	65.3 ± 11.3 (range 47-79)	60.4 ± 14.0 (range 37-84)	.319
Male/female	3/8	6/20	.786
BMI, kg/m^2	25.6 ± 2.1	26.0 ± 0.9	.832
Time since diagnosis, months	10 (IQR 2-69)	7 (IQR 2-51)	.682
Urolithiasis, n (%)	1 (9.0)	5 (19.2)	
Current medication use			
Antiresorptives, n (%)	3 (27.3)	6 (23.1)	.786
Calcimimetics, n (%)	0 (0.0)	7 (26.9)	.080
Calcium[a], mmol/L	2.7 ± 0.1	2.7 ± 0.2	.600
PTH[b], pmol/L	11.0 ± 0.8	10.0 ± 2.3	.544
Diagnosis by DXA			.057
Normal BMD, n (%)	1 (9.1)	5 (19.2)	
Osteopenia, n (%)	4 (36.4)	17 (65.4)	
Osteoporosis, n (%)	6 (54.5)	4 (15.4)	
LS BMD, g/cm2	0.86 ± 0.16	0.94 ± 0.14	.115
T-Score LS	−1.6 ± 1.3	−0.9 ± 1.3	.170
FN BMD, g/cm^2	0.62 ± 0.08	0.73 ± 0.11	**.007**
T-Score FN	−2.2 ± 0.7	−1.2 ± 1.0	**.009**
BMSi	**74.7 ± 6.0**	**79.6 ± 5.0**	**.015**

Values are expressed as mean ± SD. BMI and PTH are expressed as median ± SEM. *P* values in bold are significant. Abbreviations: BMD, bone mineral density; BMSi, Bone Material Strength index; DXA, dual x-ray absorptiometry; FN, femoral neck; LS, lumbar spine; NHNV, nonhip nonvertebral; PTH, parathyroid hormone. [a]Calcium (albumin-corrected) reference range, 2.15-2.55 mmol/L. [b]PTH reference range, 0.7-8.0 pmol/L.

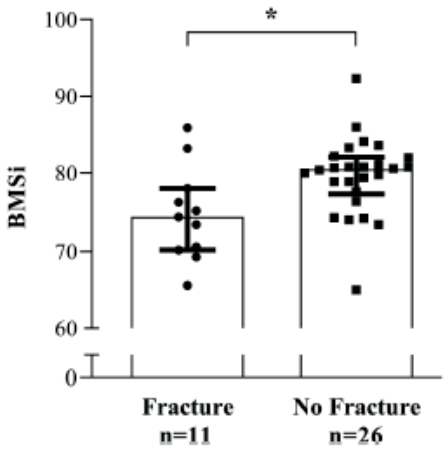

Figure 3. Bone Material Strength index (BMSi) in patients with PHPT with and without fragility fractures. Data are shown in box and whisker plots and statistical differences are displayed for BMSi. Boxes indicate median and interquartile range. Bars indicate minimum and maximum values. *****P = .015.

Discussion

We show here that BMSi values, measured by in vivo IMI, are significantly lower in patients with PHPT than in euparathyroid matched controls, independently of BMD values. Moreover, BMSi values were significantly lower in patients with PHPT with prevalent fragility fractures than in patients with PHPT without fragility fractures, and inversely related to disease severity.

Osteoporosis and fragility fractures are a known complication of PHPT and both are an indication for surgical treatment of the disease (1-3). The reported prevalence of fragility fractures in PHPT is high, 21% for vertebral fractures in a recent paper (17) as also seen in our data: Almost 30% of PHPT patients had sustained a fragility fracture, of whom 15% had a prevalent vertebral fracture. Of note, DXA T-scores of PHPT patients were in the osteopenic range, and only FN BMD, but not LS BMD, differed between fracture and nonfracture PHPT patients. This is consistent with findings from other studies, showing that bone mineral density typically is affected at sites rich for cortical bone as the FN, and relatively normal at sites rich for trabecular bone such as the spine (3). The increased fracture rate in the presence of only mildly decreased FN BMD and relatively normal lumbar spine BMD suggests that in PHPT components of bone quality other than bone mass may be altered, such as bone microarchitecture or bone material properties.

Our results indeed show a lower BMSi in patients with PHPT than in euparathyroid controls, independently of BMD values, indicating that next to BMD bone material properties are affected in PHPT. This is in agreement with a preliminary report by Starr et al. published only in abstract form in which BMSi was measured in 13 subjects with PHPT and shown to be significantly lower than that of 22 euparathyroid matched controls, 67.8 ± 9 vs 77.2 ± 8, $P = .01$ (18). Interestingly, BMSi values of their younger controls were similar to the BMSi values of our PHPT patients, 11 of whom had fractures. Fracture data or relation with disease severity, however, were not reported.

In addition, we found that BMSi was lower in patients with PHPT with several types of fragility fractures than in patients with PHPT without fragility fractures, also independently of BMD values. This has not been reported before in patients with PHPT but is in keeping with the results of most studies in patients with primary osteoporosis in which BMSi was found to be lower in patients with fragility fractures than in patients without fragility fractures, independently of BMD values (7-10). Of note, although BMSi is measured at the tibia, a site rich in cortical bone, earlier studies from our group and others in patients with primary osteoporosis and fragility frac-

tures suggest that low BMSi is associated with increased bone fragility at all relevant skeletal sites, vertebral, nonvertebral, and hip sites (8, 9, 19).

Moreover, BMSi of PHPT patients was also lower in patients with known longer duration of the disease, and in patients with higher PTH and calcium values, suggesting that material properties of bone are more severely altered the longer the exposure to elevated PTH levels and more severely uncontrolled the disease. The relation between PTH and BMSi has never been studied in hyperparathyroidism but it has been reported in a recent study by Starr et al. in 17 patients with hypoparathyroidism and 17 matched euparathyroid controls (20). It was observed that euparathyroid controls with higher PTH levels had higher BMSi values, suggesting that PTH levels might be associated with BMSi as in our study, although reversed, r = 0.58, *P* = .02. Numbers of our study and their study, however, are small.

Our study has strengths as well as limitations. One of the strengths is the consecutive inclusion of men and women with PHPT with and without fragility fractures at a tertiary referral center, giving a representative image of the whole PHPT patient population in clinical practice, and the possibility for proper matching. An additional interesting BMD measurement site would have been the distal 1/3 radius, a location known to be affected by PHPT. Unfortunately due to logistic reasons this was not possible. Nevertheless, BMD data were available from all PHPT and controls at the FN and LS, and no correlation between BMSi and BMD was found. Another limitation is the inclusion of patients with PHPT also treated with antiresorptives and/or cinacalcet. Another study performed by our group demonstrated that treatment with bisphosphonates and denosumab, given for a mean period of 2 years, increases BMSi values in patients with low bone mass and increased risk of fracture (15). While that study was performed in patients with low bone mass and increased risk of fracture in whom treatment with antiresorptives has been shown to be associated with a decrease in fracture risk (21-23), this has not been shown in patients with PHPT (1). Also, when assuming an increase in BMSi in patients with PHPT treated with bone agents the difference in BMSi between patients and controls who were treatment naive is even more remarkable. Moreover, we found no significant difference in BMSi between patients with PHPT treated with antiresorptives for a median duration of 2.5 years and treatment-naive patients. In addition, repeated analysis in only treatment-naive patients with PHPT did not change results, and sample size calculation was based on pilot data also including treated patients.

In conclusion, we show that BMSi is lower in patients with PHPT than in matched euparathyroid controls, independently of BMD, and that BMSi is the lowest in patients with PHPT and a fragility fracture. These findings indicate that tissue-level properties of bone are impaired in patients with PHPT. Our results might thus help

explain the higher fracture risk than expected from measurements of BMD in PHPT probably at both at cortical and at trabecular sites. Therefore, and since the IMI technique enables physicians to evaluate in vivo tissue level material properties of bone in a minimally invasive, simple, and safe manner (6), it has the potential to be used as an additional tool to DXA BMD measurements in the evaluation of bone fragility in patients with PHPT in the clinic, although larger prospective studies are warranted.

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Chapter 6

Bone Material Strength Index Is Low in Patients With Cushing's Syndrome Even After Long-term Remission

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Abstract

Objective: Hypercortisolism in endogenous Cushing's syndrome (CS) results in decreased bone mineral density (BMD) and increased fracture risk. Although after remission BMD improves, the fracture rate remains elevated, suggesting that BMD may not adequately reflect fracture risk in this group. The aim was to evaluate bone material properties, another component of bone quality, using impact microindentation in patients with CS in remission.

Methods: Cross-sectional study in 60 CS patients and 60 age-, sex-, and BMD-matched controls at a tertiary referral center between 2019 and 2021. Bone material strength index (BMSi) was measured by impact microindentation using the OsteoProbe® device at the tibia. In addition, laboratory investigation, BMD, and vertebral fracture assessment were performed.

Results: By design, patients and controls were comparable for age (median age 56.5 years), sex (48 women), and BMD at the lumbar spine and femoral neck. They were also comparable regarding the number of fragility fractures (21 vs 27, $P = .22$). The median time of remission in patients was 6 years (range 1 to 41). Despite comparable BMD, BMSi was significantly lower in CS patients compared to controls (76.2 ± 6.7 *vs* 80.5 ± 4.9, $P < .001$). In CS patients, BMSi was negatively correlated with body mass index ($r = -0.354$, $P = .01$) but not related to the presence of fracture, physiological hydrocortisone replacement use, other pituitary insufficiencies, or time since remission.

Conclusion: Bone material properties remain altered in patients with endogenous CS, even after long-term remission. These abnormalities, known to be associated with fractures in other populations, may play a role in the persistent bone fragility of steroid excess.

Introduction

Cushing's syndrome (CS) is a rare endocrine condition characterized by endogenous glucocorticoid excess, resulting from ACTH-secreting pituitary adenoma, ectopic ACTH production, or autonomous adrenal cortisol overproduction. Osteoporosis and fragility fractures are a main clinical manifestation of the disease and a relevant cause of morbidity and mortality (1-3). The skeletal complications are characterized by decreased bone formation and increased bone resorption, resulting in bone loss and an increased fracture risk. At diagnosis, osteoporosis is observed in up to 50%, and fractures have been reported in 30% to 76% of patients (3, 4). Fractures typically also occur in patients with normal or only slightly decreased bone mineral density (BMD) (5), suggesting that in CS factors contributing to bone quality other than BMD are also affected and have to be taken into account when assessing fracture risk in this group. Moreover, following remission of hypercortisolism, BMD increases toward values in the normal range, but fracture risk seems to remain increased (6-9).

Factors contributing to bone quality include bone architecture on the macro and micro level and tissue material properties (10). The microarchitecture of cortical and trabecular bone at the radius and the tibia has been assessed in 30 patients with active endogenous CS using high-resolution peripheral quantitative computed tomography and found to be altered compared to healthy controls (11). Tissue material properties of bone, however, could only be assessed until recently ex vivo by using transiliac bone biopsy specimens. Since the introduction of the impact microindentation (IMI) technique, these properties have become measurable in humans in vivo (12). IMI is performed with the handheld device OsteoProbe® that imparts a single impact load to the bone surface and is approved for use at the tibia. By driving the probe into the bone surface, the resistance of bone tissue to a given mechanical challenge can be measured as the bone material strength index (BMSi). BMSi values are altered in patients with fragility fractures compared to patients without fractures, independently of BMD (13-16), and are strongly associated with material properties of subperiostal mineralized bone surface (17). In a study conducted in patients requiring exogenous glucocorticoid therapy for various underlying disorders, the BMSi was found to be significantly decreased already after 7 weeks of treatment (18).

To date, there is no data on BMSi measurements in patients with endogenous CS. Therefore, the aim of this study was to evaluate whether bone material properties, as assessed by IMI, are altered in patients with endogenous CS in remission compared to controls in order to provide additional information on bone health in this group.

Patients and Methods

Study Design

This is a cross-sectional study evaluating BMSi in men and women in remission after treatment for CS and in age-, sex-, and BMD-matched controls. All BMD ranges and patients with and without fragility fractures were included. Patients and controls were studied at the outpatient clinics of the Leiden University Medical Center (LUMC), the coordinating center of the European Reference Network for Rare Endocrine conditions, and a European reference center for pituitary and adrenal diseases.

The study was approved by the institutional medical ethical committee, and all subjects provided full, written informed consent. All procedures performed were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Patients in Remission After Treatment for CS

The whole cohort of patients aged 18 to 85 years with adult-onset CS that were in remission for at least 6 months and under periodical prospective follow-up at the outpatient clinic of the Department of Endocrinology of the LUMC since 1978 (19) were eligible to participate in the study. The cohort patients were included between June 2019 and August 2021; the inclusion period was prolonged due to the COVID-19 pandemic. The diagnosis of CS as well as treatment of these patients including surgery, preoperative cortisol-lowering treatment as needed, posttreatment hydrocortisone (HC) replacement therapy, and tapering when the axis improved followed the Endocrine Society clinical practice guidelines and their update by the Pituitary Society (20, 21). The state of remission was also established according to these guidelines and was defined both as insufficient cortisol secretion after treatment of hypercortisolism with the need for hydrocortisone replacement therapy and, in the case of presumed normalized cortisol secretion, as normal 24-hour urinary free cortisol (UFC), midnight salivary cortisol, and normal overnight 1 mg dexamethasone suppression test.

Controls

Men and women who had no history of CS or exogenous steroid excess served as controls. They were recruited from the outpatient clinics of the Center for Bone Quality or from the regional Fracture Liaison Service of the LUMC and were matched for age, sex, and BMD.

Exclusion criteria for both groups were: the presence of a metabolic bone disease other than osteoporosis, any untreated endocrine disorder, severe liver insufficiency or chronic kidney disease (stage IV or V), immobilization, a contraindication for IMI measurement (22), or inability to provide informed consent. In addition, the use of any treatment affecting bone metabolism, other than calcium and vitamin D, was also an exclusion criterion, with the exception of bisphosphonate (BP) or denosumab use in patients in remission after treatment for CS (CS patients). The presence or absence of fragility fractures was not considered in the selection process of controls.

Methods

A full medical history; use of medication including agents affecting bone metabolism, vitamin D supplementation, and dietary calcium intake; and a detailed fracture history with documentation of sites and dates of occurrence of fractures were obtained from all subjects. A fragility fracture was defined as any low-energy fracture, excluding those of the hands, feet, and skull. Data on clinical risk factors for fracture as used in the FRAX algorithm were obtained from all subjects (23). In CS patients, clinically relevant data regarding hypercortisolism, such as date of surgery, number of relapses, date of remission after last recurrence, HC dependency, and postsurgical pituitary hormone deficiencies were also retrieved from the patient files. Duration of HC replacement was defined as the period of time that patients required HC replacement; in those currently on HC replacement, duration was calculated from the initiation of replacement therapy until the date of IMI performance.

Laboratory Parameters

Serum calcium (albumin-corrected) and creatinine concentrations were measured by semiautomated techniques. Plasma intact PTH was measured using the Immulite 2500 (Siemens Diagnostics, Breda, The Netherlands) and serum 25-hydroxyvitamin D concentrations by the 25- hydroxyvitamin-vitamin D TOTAL assay (DiaSorin D.A./N.V., Brussels, Belgium). In CS patients, clinical remission was biochemically confirmed by the assessment of UFC, serum, and salivary cortisol. UFC was analyzed using an in-house LC-MS/MS method, calibrated using Cerilliant certified reference material C-106, cortisol 1mg/mL in methanol. Twenty-four-hour cortisol concentrations below 150 nmol/24 hours were considered normal. Serum and salivary cortisol were analyzed using a Roche ECLIA Cortisol assay (second generation) on a Modular E170 immunoanalyzer (Roche Holding AG, Basel, Switzerland). In midnight saliva, cortisol levels below 7.5 nmol/L were considered normal (21). The cutoff limit for the dexamethasone suppression test was 50 nmol/L (21).

BMD

Areal BMD was assessed at the lumbar spine (L1-L4) and both hips using dual-energy X-ray absorptiometry (DXA) performed with the Hologic QDR Discovery A machine (Hologic, Bedford, MA, USA). The mean BMD values of the femoral neck were utilized for the analysis. Z-scores were calculated using National Health and Nutrition Examination Survey III reference values compatible with the reference values of the Dutch population (24).

Vertebral Fracture Screening

At the time of inclusion, single energy X-ray lateral vertebral fracture assessment images of the spine (T4-L4) were obtained through DXA or conventional antero-posterior and lateral radiographs of the thoracic and lumbar spine, adhering to standardized protocols for vertebral deformity detection. These images were then evaluated independently by 2 experienced readers using the semiquantitative Genant method (25). All vertebral fractures were considered in the analysis.

IMI

BMSi was assessed in all patients using the handheld microindenter device (OsteoProbe® RUO, Active Life Scientific, CA, USA) by IMI at the midshaft of the tibia, as described previously (26). Briefly, patients were positioned in a supine position with the tibia externally rotated to align the flat surface of the medial tibia diaphysis in a horizontal position. The measurement site was defined as the mean distance between the medial malleolus and the distal apex of the patella. After disinfection and local anesthesia of the skin and periosteum with lidocaine 1%, the test probe was gently inserted into the skin until reaching the bone surface. Following at least 5 valid measurements, an additional 5 measurements were taken on a polymethylmethacrylate calibration phantom. BMSi was computed by the computer software. Three experienced operators conducted the measurements. Intraobserver and interobserver coefficients of variation in our center were found to be 2.2% and 1.6%, respectively.

Statistical Analysis

Results are presented as mean ± SD unless stated otherwise. Descriptive statistics were used to describe clinical and laboratory parameters. Normality assumptions were checked by inspection of histograms and tested by a Kolmogorov–Smirnov test. Differences in baseline characteristics between patients and controls were assessed using a paired *t*-test or Wilcoxon signed ranks test and McNemar's test for normally and not normally distributed continuous and for categorical variables, respectively. Conditional logistic regression was used to assess BMSi and BMD values adjusted for body mass index (BMI) to compare BMSi and BMD values between patients and controls. Correlations between BMSi values and patients' or disease characteristics were examined by Pearson's and Spearman's tests for normally and

not normally distributed variables, respectively. A *P*-value <.05 was considered to be statistically significant. All analyses were performed using SPSS software for Windows (version 29.0; SPSS Inc., Chicago, IL, USA) and graphs were constructed with Graphpad Prism (version 8.0; GraphPad Software Inc., La Jolla, CA, USA).

Results

Sixty-one of 73 eligible patients with CS in remission agreed to participate (Fig. 1). The IMI measurement could not be performed in 1 patient due to excessive lower leg edema, resulting in 60 CS patients included in the study (Table 1). Of these, 48 were women and 12 were men, with a median age of 56.5 years (range 25-76 years). The median age at diagnosis was 40.0 years (range 18-66 years). A corticotroph pituitary adenoma was the main cause of endogenous hypercortisolism in most patients (n = 51), followed by adrenal (n = 7) and ectopic origin (n = 2). The median duration of remission was 6.0 years (range 1-41 years). Twenty-one patients (35%) had recurrent hypercortisolism requiring reintervention before remission was achieved (range 1-4 recurrences). Among the 48 women, 30 (62.5%) were premenopausal and 18 were postmenopausal at the time of remission. The median time since menopause at the time of IMI measurement was 11.5 years (range 1-27 years).

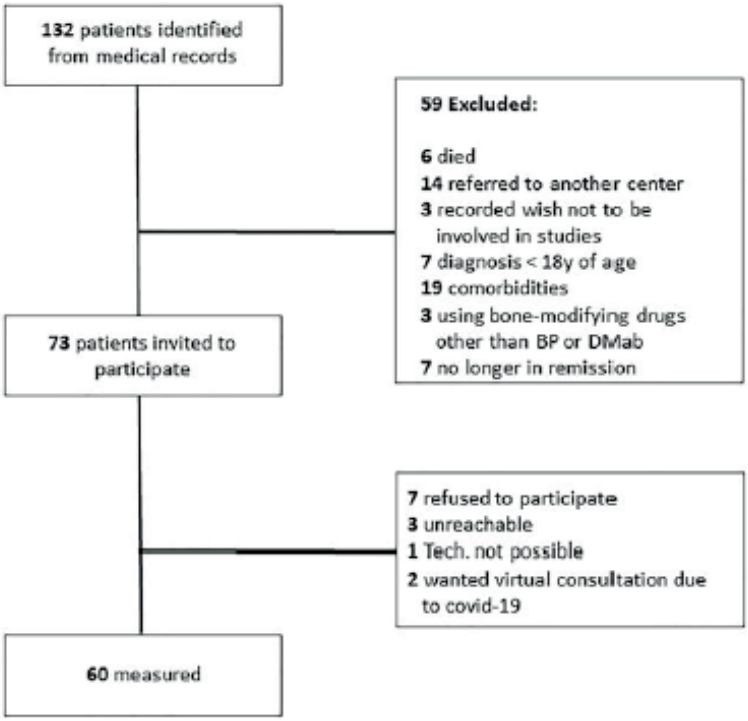

Figure 1. Patient flowchart.

Table 1. Disease-specific characteristics of patients with CS in remission

Characteristics n	CS in remission 60
Age at diagnosis, years (range)	40.0 (18-66)
Time since diagnosis, years (range)	8.4 (1-42)
Origin of hypercortisolism, n (%)	
Pituitary	51 (85.0)
Adrenal	7 (11.7)
Ectopic	2 (3.3)
UFC at diagnosis (nmol/24 hours)[a]	646 (25-7792)
Recurrence before final remission, n (%)	21 (35.0)
Current HC replacement, n (%)	30 (50.0)
Current HC dosage, mg	20.0 (5-30)
Current hypopituitarism,n (%)[b]	
Any hypopituitarism	21 (41.2)
Hypothyroidism	19 (37.3)
Hypogonadism	12 (23.5)
GH deficiency	15 (29.4)
Current laboratory values	17.0 ± 3.0
Free T4 (pmol/L)[c]	12.4 (0.1-78.5)
LH (IU/L)[d]	13.0 (0.1-101.5)
FSH (IU/L)[e]	18.8 ± 6.5

Values are expressed as median (ranges). Free T4 and IGF1 are expressed as mean ± SD. Abbreviations: CS, Cushing's syndrome; HC, hydrocortisone; UFC, urinary free cortisol. [a]UFC was available in 49 patients. [b]Percentage expressed for all patients with pituitary hypercortisolism only. *c*Free T4 reference range, 12-22 pmol/L. *d*LH reference range, premenopausal 1-60 IU/L; postmenopausal 8-60 IU/L, men 2-9 IU/L. *e*FSH reference range, premenopausal 2-21.5 IU/L; postmenopausal 26-135 IU/ L, men 1.5-12.5.

After remission, 53 patients (88.3%) had been HC dependent, of which 30 patients (50%) were still using HC replacement at the time of the BMSi measurement. The median HC dosage was 20 mg per day (range 5-30 mg). Of the 51 patients treated for Cushing's disease, 21 (41.2%) also had other pituitary hormone deficiencies. Central hypothyroidism was reported in 19 (37.3%) and adequately substituted in all of them. Hypogonadotropic hypogonadism was present in 12 (23.5%) and GH deficiency in 15 patients (29.4%).

At the time of inclusion, 7 patients (11.7%) were using antiresorptive agents (5 oral BP, 1 IV BP, 1 denosumab) for a median duration of 37 months (range 13-55 months). Reasons for treatment with antiresorptive agents were osteoporosis or osteopenia with and without fractures (n = 5) and to decrease fracture risk in the presence of severe CS (n = 1). In 1 patient with normal BMD, antiresorptive treatment had been initiated due to the occurrence of multiple vertebral fragility fractures leading to

Table 2. Characteristics of patients with CS in remission and controls

	CS in remission (n = 60)	Controls (n = 60)	*P*-value
Age, years	56.5 (25-76)	56.5 (20-74)	.97
Male/female	12/48	12/48	1.00
LS BMD, g/cm²	0.96 ± 0.13	0.96 ± 0.13	.82
Z-score LS	0.3 ± 1.2	0.2 ± 1.2	.64
FN BMD, g/cm²	0.75 ± 0.12	0.74 ± 0.10	.70
Z-score FN	−0.1 ± 0.9	0.0 ± 0.9	.82
BMI, kg/m²	26.1 (18.7-43.0)	25.4 (14.7-36.5)	.09
Smoking, n (%)	11 (18.3)	11 (18.3)	1.00
Alcohol use ≥ 3 U/day, n (%)	4 (6.7)	8 (13.3)	.22
Calcium, mmol/L[a]	2.4 ± 0.1	2.3 ± 0.1	<.001
Creatinine,umol/L[b]	74.3 ± 15.6	69.0 ± 13.3	.05
25-OH vitamin D,nmol/L[c]	82.7 ± 29.6	71.1 ± 28.9	.03
PTH, pmol/L,pmol/L[d]	4.8 (2.4-13.8)	2.8 (1.0-7.5)	<.001
Fragility fracture any, n (%)[e]	21 (36.2)	27 (47.3)	.22
History of hip fracture, n (%)	0 (0.0)	5 (8.3)	.01
Prevalent vertebral fracture (radiological), n (%)[f]	15 (25.9)	6 (10.5)	.03
History of NHNV fracture, n (%)	11 (18.3)	20 (33.3)	.06
BMSi	76.2 ± 6.7	80.5 ± 4.9	<.001

Values are expressed as mean ± SD. Age, BMI, and PTH are expressed as median and ranges. Bold numbers indicate statistical significance. Abbreviations: BMD, bone mineral density; BMI, body mass index; BMSi, bone material strength index; CS, Cushing's syndrome; FN, femoral neck; LS, lumbar spine; NHNV, nonhip nonvertebral. [a]Calcium (albumin-corrected) reference range, 2.15-2.55 mmol/L. [b]Creatinine reference range, 64-104 umol/L for males; 49-90 umol/L for females. [c]25-OH vitamin D reference range, 50-250 nmol/L. [d]PTH reference range, 0.7-8.0 pmol/L. [e]Percentages were calculated for 58 patients and 57 controls. [f]Vertebral fracture screening was available in 57 patients and 56 controls.

transient paraparesis 3 years after remission of CS. None of the patients was using osteoanabolic agents.

Patients' characteristics and laboratory test results of the 60 CS patients (48 women) and 60 matched controls (48 women) are shown in Table 2. By design, the 2 groups were comparable for age, sex, and BMD at the lumbar spine (LS) and femoral neck (FN). They were also comparable regarding the number of fragility fractures. A significantly higher number of patients were using vitamin D compared to controls (n = 36 vs n = 20, *P* = .006), while there was no difference in the number of patients and controls using oral calcium supplements (n = 18 vs n = 12, *P* = .225). The majority of patients and controls had osteopenia (53.3% and 60%), while 23 patients and 19 controls had normal BMD, and 5 patients and controls had osteoporosis, respectively.

BMSi

BMSi values were significantly lower in CS patients compared to controls, 76.2 ± 6.7 vs 80.5 ± 4.9, *P* < .001 (Fig. 2), also after the exclusion of the 7 patients using antiresorptive agents (*P* < .001). BMSi values of the 2 patients with ectopic CS were very low, at 66.2 and 69.2 (*P*-value not available due to the low number of patients), while mean values of patients with pituitary and adrenal CS were comparable: 76.6 ± 7.0 vs 76.0 ± 3.1, *P* = .686. However, the findings of this study did not change when excluding the 2 patients with ectopic CS.

In CS patients, BMSi values negatively correlated with BMI (r = −0.354, *P* = .006), while in controls this correlation was absent (r = −0.135, *P* = .304). BMSi values did not correlate with age (r = 0.160, *P* = .223), LS BMD (r = 0.102, *P* = .461), or FN BMD (r = 0.077 *P* = .557) in patients or controls, although in controls there was a borderline significant weak correlation with age (r = −0.246, *P* = .058).

There was no correlation of BMSi with age at diagnosis (r = 0.184, *P* = .159), but the 2 lowest BMSi results (BMSi 60.7 and 60.9; see Fig. 2) were found in the 2 patients who were the youngest at the time of diagnosis (18 and 20 years). There was no correlation of BMSi with UFC levels at diagnosis (r = 0.061, *P* = .676) or with the time since disease remission (r = −0.087, *P* = .510), including when stratified by remission intervals of 5 years (0-5 years, 6-10 years, 11-15 years, etc.).

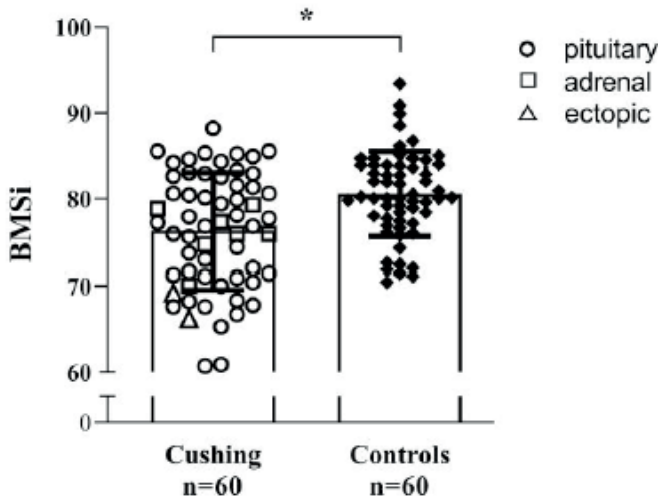

Figure 2. BMSi in Cushing patients and controls. Data are shown in box-whisker plots and statistical differences are displayed for BMSi. Boxes indicate the median and interquartile range. Bars indicate minimum and maximum values. **P* < .001.

BMSi values in CS (excluding the outliers with ectopic Cushing's) differed between women and men (75.6 ± 6.5 vs 80.5 ± 5.7, *P* = .032), which was not the case in female and male controls (80.1 ± 4.5 vs 82.0 ± 6.5, *P* = .611). BMSi values of the 30 women who achieved remission during premenopause did not significantly differ from those of the 18 women who achieved remission during postmenopause (74.8 ± 6.9 vs 76.6 ± 6.0, *P* = .443). HC dependency at the time of inclusion did not affect BMSi values: they were comparable in 30 patients who were HC dependent compared to those in 30 patients who were not HC dependent (75.9 ± 7.1 vs 76.5 ± 6.3, *P* = .731), also after adjusting for BMI (*P* = .761). BMSi values did not correlate with the daily HC dose used (r = −0.266, *P* = .172), nor did it correlate in the 57 patients using physiological doses up to 20 mg per day. However, the mean BMSi of the 3 patients using supraphysiological HC doses (>20 mg/day) was very low, 72.9 ± 8.7, and numerically strikingly lower than the mean BMSi of the patients using physiological doses, 76.8 ± 7.2 (*P*-value not available due to the low number of patients using supraphysiological doses). BMSi values did not differ between patients with and those without other pituitary hormone deficiencies (data not shown).

BMSi, BMD and Fractures

Twenty-one patients (35%) with CS in remission had previously experienced 1 or more fragility fractures, including those detected on spine images. Of these, 15 had vertebral fractures, 6 had both vertebral and nonvertebral fractures, and 11 patients had nonvertebral fractures only. Of the 15 patients with vertebral fractures, 7 patients had multiple vertebral fractures (range 2-10). In total, 4 grade 3 vertebral fractures, 16 grade 2 vertebral fractures, and 21 grade 1 vertebral fractures were detected. Clinical vertebral fractures were diagnosed in only 2 patients, while in 13 they were detected radiologically.

Two CS patients and 3 controls, who had no other fragility fractures, did not undergo vertebral fracture screening and were consequently excluded from further analysis, as they could not be accurately categorized as "nonfractured." As a result, the final analysis included 58 CS patients and 57 controls.

The baseline characteristics and laboratory measurements of CS patients with and without fractures were comparable (see Table 3). BMSi did not differ between patients with and patients without fractures (77.0 ± 7.1 vs 75.9 ± 6.5, *P* = .551), also after adjusting for BMI (*P* = .139), sex (*P* = .641), or age (0.575) or when analyzing men and women separately (data not shown). BMSi did also not differ between the 15 patients with vertebral fractures and the 43 patients without vertebral fractures (77.7 ± 5.7 vs 75.8 ± 7.0, *P* = .321).

In contrast, in controls, BMSi was significantly lower in those with fragility fractures (n = 27) compared to those without fractures (n = 30) (77.7 ± 4.2 vs 82.9 ± 4.4, *P* < .001), also after adjusting for sex (*P* < .001) or age (*P* < .001). BMSi values of controls with fragility fractures were also comparable to BMSi values of CS patients with fragility fractures (77.7 ± 4.2 vs 77.0 ± 7.1, *P* = .934).

Table 3. Characteristics of CS patients with and without fragility fractures

	Fracture (n = 21)	**No fracture (n =)**	***P*-value**
Age, years	56.0 (31-76)	56.0 (25-73)	.71
Male/female	5/16	6/31	.48
LS BMD, g/cm²	0.98 ± 0.12	0.95 ± 0.13	.34
Z-Score LS	0.6 ± 1.1	0.1 ± 1.3	.20
FN BMD, g/cm²	0.75 ± 0.12	0.74 ± 0.12	.85
Z-score FN	0.0 ± 0.8	−0.1 ± 1.0	.80
BMI, kg/m²	27.3 (18.7-43.0)	25.3 (19.7-39.4)	.09
Smoking, n (%)	4 (19.0)	7 (18.9)	.99
Alcohol use ≥3 U/day, n (%)	3 (14.3)	1 (2.7)	.09
Calcium,, mmol/L[a]	2.4 ± 0.1	2.4 ± 0.1	.69
Creatinine,umol/L[b]	74.1 ± 12.4	74.9 ± 17.6	.85
25-OH vitamin D,nmol/L[c]	89.5 ± 37.2	78.9 ± 22.7	.20
PTH, pmol/L,pmol/L[d]	4.6 (3.1-13.8)	4.7 (2.4-11.0)	.63
UFC (nmol/24 hours)[e]	693 (60-7792)	641 (25-4991)	.50
BMSi	77.0 ± 7.1	75.9 ± 6.5	0.55

Values are expressed as mean ± SD. Age, BMI, PTH, and UFC are expressed as median and ranges. Bold numbers indicate statistical significance. Abbreviations: BMD, bone mineral density; BMI, body mass index; BMSi, bone material strength index; FN, femoral neck; LS, lumbar spine; UFC, urinary free cortisol. [a]Calcium (albumin-corrected) reference range, 2.15-2.55 mmol/L. [b]Creatinine reference range, 64-104 umol/L for males; 49-90 umol/L for females. [c]25-OH vitamin D reference range, 50-250 nmol/L. [d]PTH reference range, 0.7-8.0 pmol/L. [e]UFC was available in 20 patients with and 28 patients without fractures.

In CS patients, BMD did not differ between patients with and patients without fractures either at the LS or at the FN (LS: 0.98 ± 0.11 vs 0.95 ± 0.14, *P* = .367; FN 0.75 ± 0.12 vs 0.75 ± 0.12, *P* = 0.951). A DXA diagnosis of osteoporosis was established in only 1 of the patients with fractures, while 9 had osteopenia and 8 normal BMD, respectively.

All findings of this study did not change when excluding the 7 patients currently using antiresorptive agents.

Discussion

This study demonstrates that BMSi values, measured by IMI at the tibia, are significantly lower in patients in remission after treatment for CS compared to matched controls and are not correlated to BMD values. In this cross-sectional study, there is no difference in BMSi values in CS patients with compared to those without fragility fractures, which is in contrast to controls in whom BMSi values are lower in those with fractures. In addition, BMSi values are negatively correlated to BMI in CS patients only, which might reflect the severity of cortisol exposure during active disease. Low BMSi values, known to be associated with prevalent fractures in other populations, may thus at least in part play a role in the observed persistent bone fragility in patients treated for CS.

Osteoporosis and fragility fractures are a known complication of CS. Osteoporosis is present in up to 50% at diagnosis (3, 11, 27-29), and the reported prevalence of fragility fractures in CS in the literature is high, ranging from 30% to 76% (3, 4). As shown in several studies, BMD clearly increases and usually returns to normal values after successful abrogation of cortisol excess (6-9, 30-34), although large-scale longitudinal studies are lacking. Also in our study, mean DXA Z-scores after a mean of 6 years after remission were in the normal range (+ 0.3 SD at the LS and −0.1 SD at the FN, respectively). Fracture risk is highest within the last 2 to 3 years before diagnosis of CS and seems to decrease after treatment of the disease (1, 9, 35, 36). However, scarce data indicate that fracture risk might remain increased up to 30 years after diagnosis (1, 3, 37).

This is to our knowledge the first study reporting BMSi values, measured by IMI, in patients with CS. Our results indeed show lower BMSi values in CS patients 6 years after remission, compared to matched controls, in the presence of comparable BMD values. Mean BMSi values of our CS cohort after long-term remission were comparably low to those measured in patients exposed to exogenous glucocorticoids, known to rapidly increase fracture risk, for various underlying disorders after 20 weeks of glucocorticoid-treatment (median BMSi 77.3) (18). Half of our cohort of CS patients was using oral HC replacement at a physiological dose at the time of measurement, and BMSi values did not significantly differ between those who did and those who did not. The median daily HC dose used in our cohort was 20 mg, which is expected not to have adverse glucocorticoid-related effects (38, 39), but BMSi values of the 3 patients using supraphysiological doses indeed were strikingly lower.

Interestingly, we found a negative relationship of BMSi with BMI in our CS cohort, meaning that patients with higher BMI had lower and thus worse BMSi values. Both higher BMI and lower BMSi could be consequences of more severe cortisol exposure

during active disease. This is supported by the fact that there was no correlation between BMSi with BMI in controls, as in earlier published studies by our group performed in patients with primary osteoporosis with or without fractures and in patients with acromegaly (13, 40) and with the fact that the usually observed correlation of BMSi with age was not present, although data in the literature on the correlation of BMSi with BMI or age are conflicting (12). However, we found no correlation of BMSi with 24-hour UFC concentrations as a marker of disease severity at the time of diagnosis, although waist circumference, or the Cushing severity index, which was first described in 2000 and thus after our cohort started, would be better markers of disease activity (41). These clinical data have only inconsistently been recorded, and prospective studies designed to answer this question would be needed. There might also be a negative influence of adipose tissue itself on bone strength, as was suggested by a study performed in elderly Swedish women. That study reported an inverse relationship between BMSi and the amount of subcutaneous fat at the tibia, whole body fat mass, and BMI (42). A third explanation would be a more metrological one, hypothesizing that subjects with higher BMI have more adipose tissue at the tibia, which itself could interact with the IMI measurement since the needle has to stick through a greater amount of tissue and thereby possibly generates lower BMSi values. However, the latter 2 hypotheses would also apply to controls, among whom no correlation of BMSi with BMI was observed.

Also, after adjusting for BMI, BMSi but also BMD values did not differ between CS patients with prevalent fractures and those without. Thirty-five percent of our CS patients had fractures, which is in accordance with previously reported rates (3, 7, 37). Vertebral fractures were present in 20% of our cohort. However, the majority of those fractures had been detected radiologically only, and routine screening for vertebral fractures was not conducted for all patients at the time of diagnosis. Therefore, it cannot be ruled out that some vertebral fractures occurred during active disease or shortly after remission, when BMSi may have been different. Furthermore, some patients had been treated with antiresorptive agents during active but also remitted disease, which might have influenced their BMSi values. Although CS patients using antiresorptive agents at the time of IMI measurement had comparable BMSi values to those using none, another study performed by our group demonstrated that antiresorptive treatment, given for a mean period of 2 years, increases BMSi values in patients with low bone mass at risk for fracture (26). In contrast, in control subjects, BMSi was lower in patients with fractures compared to those without fractures. This is in accordance with the results of many earlier studies, in which subjects with fractures (hip, vertebral, nonhip-nonvertebral) had lower BMSi values compared to adequate controls without fractures (13-16, 43-45). These earlier studies suggest that BMSi measured at the tibia is associated with increased bone fragility at all relevant skeletal sites. Strikingly, the mean BMSi values of our whole CS cohort

were in the range or even lower than those of most reported fracture groups and also were lower than the very recently published mean reference values of healthy women and men of comparable age from Australia, Europe, and the United States (mean BMSi 81.3) (46). The finding that female CS patients had lower BMSi values than male CS patients is somewhat surprising. Some earlier studies on bone health in CS patients revealed rather higher BMD values and a lower fracture rate in female compared to male patients (6, 9, 28, 47, 48). In addition, most earlier studies on BMSi did not show any differences between women and men (12). However, the aforementioned very recent publication on reference intervals in healthy adults also including healthy participants from our center reported lower BMSi for women (79.0 ± 9.1) than for men (84.4 ± 6.9), which might very well also apply to our CS patients, independently of their disease (46). Furthermore, a very recent study in patients with adrenal (subclinical) CS also detected an increased fracture incidence in postmenopausal women (49).

Our data thus highlight that the follow-up of patients with CS—not only of those with active disease but also of those in remission—should focus on bone health as well. In addition to fracture history, DXA BMD measurement, and ensuring adequate calcium and vitamin D substitution when necessary, a vertebral fracture screening using vertebral fracture assessment or spine radiographs should also be performed in all patients, regardless of disease activity. Vertebral fractures, which have been shown to be associated with high morbidity and mortality (50-54), are highly prevalent in this disease, and only half of patients present with clinical symptoms (4). Bone material properties rather than BMD might be affected after remission of the disease, as suggested by our study. The lowest BMSi values were observed in patients with ectopic CS, in patients with early onset of disease, and in those using supraphysiological HC doses, all of which are groups at risk for more severe bone involvement (2, 4, 20, 55). IMI might be a valuable additional tool in the evaluation of bone fragility in CS patients.

Our study has some limitations. First, as referenced in the Methods section, the microindentation technique is relatively novel, and reliable results can only be obtained with proper training. However, in this study, only experienced operators performed the measurements, and the inter- and intraobserver measurements were excellent. Another limitation is that our CS cohort was heterogenous with respect to time and severity of steroid exposure. Some data, such as the number of recurrences, HC use, or other pituitary hormone deficiencies, had to be collected retrospectively in some patients. However, we included a large number of participants with this rare disease, with an expected female-to-male ratio of 5:1 and a broad age range at a tertiary referral center, providing a representative sample of the whole CS in remission population in clinical practice. Furthermore, the cohort has been well documented

and followed prospectively since 1978 at the LUMC, a European reference center for pituitary and adrenal diseases. There were no missing data apart from vertebral fracture screening and 24-hour urinary cortisol for some patients at the time of CS diagnosis. Another limitation is the study's cross-sectional design, necessitated by the rarity of the disease. This design did not allow for the evaluation of BMSi at the time or prior to fracture occurrence, and it led to the inclusion of some CS patients treated with antiresorptive agents, as described previously. However, the study provides valuable information on the long-term bone sequelae of the disease. Assuming an increase in BMSi in CS patients treated with bone agents, the difference in BMSi between patients and treatment-naive controls is even more remarkable. Moreover, repeated analysis in only treatment-naive CS patients did not change results.

In conclusion, we show that BMSi values are lower in patients with endogenous CS compared to matched controls even after long-term remission and are inversely correlated with BMI in patients only. Furthermore, BMSi values are low independent of the presence of fractures, BMD values, ongoing HC use, or other pituitary hormone deficiencies. Our data thus indicate that tissue-level properties of bone are permanently impaired also after treatment of the disease. These abnormalities, which are known to be associated with fractures in other populations, may, at least in part, contribute to the persistent bone fragility in treated CS. This underscores the importance of comprehensive bone health assessment in patients with CS, not only at the time of diagnosis but also after long-term remission. Further studies on BMSi in CS patients with active disease are warranted.

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PART III:

EVALUATION OF THE SAFETY AND ACCEPTABILITY OF THE IMPACT MICROINDENTATION TECHNIQUE

Chapter 7

Safety Outcomes of Impact Microindentation: A Prospective Observational Study in the Netherlands

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ABSTRACT

Impact microindentation (IMI) is a technique to assess bone material properties of the cortical bone at the tibia in a transcutaneous, microinvasive, way. IMI is increasingly used in studies evaluating the contribution of tissue material properties to bone fragility in humans, and is approved for use in the clinic in Europe and the United States. Previous data show that IMI is well tolerated during and immediately after the procedure. The aim of this prospective observational study was to evaluate the longer-term safety and acceptability of an IMI measurement using the handheld OsteoProbe device®. Included were patients who were scheduled for a measurement at the Leiden University Medical Center from September 2019 to December 2020 and willing to participate. Patients were asked to review the procedure right after the measurement, and by telephone interviews 1 week and 1 month thereafter. The primary outcome was the 30-day complication rate after the measurement. Included were 106 patients (71 women) with a median age of 59 years (range, 20 to 86 years). Only three minor events were reported by 1-week follow-up, with an overall 30-day event rate of 2.8%. These were a very small hematoma in two patients, and a small bruise in one patient, all of which resolved without medical intervention. No other safety-related concerns were observed, and all 106 patients would undergo the measurement again if needed. The vast majority had no pain at baseline, 1-week and 1-month follow-up (80.2%, 88.4% and 94.3%, respectively). In this first and large longitudinal study we demonstrated that although minimally-invasive, IMI using the OsteoProbe® device at the tibia did not lead to any complications, and was well accepted by patients. Results strongly suggest that IMI can be safely used in studies as well as in the clinic in the hands of an experienced operator.

Introduction

The current gold standard for the diagnosis of bone fragility is the combination of clinical risk factors and a low bone mineral density (BMD) using dual-energy X-ray absorptiometry (DXA) and a vertebral fracture assessment. BMD measurements have been routinely performed in the clinic for over three decades and a low BMD is clearly associated with increased fracture risk (1). However, evidence has been accumulating over the past decades for factors contributing to bone strength other than BMD, like bone architecture on the macro and micro level, and tissue material properties (2).

Until recently, tissue material properties could only be assessed ex vivo on a transiliac bone biopsy specimen. Since the introduction of impact microindentation (IMI), the possibility has emerged for directly evaluating tissue-level properties of bone in a minimally invasive way in humans in vivo (3). With the handheld IMI device OsteoProbe®, the surface of the cortical bone at the anterior mid tibia is indented by a given impact in order to measure the resistance of bone tissue to this mechanical challenge. The resistance of bone to indentation is expressed as the bone material strength index (BMSi). The softer the bone tissue, the easier the probe indents the bone, and the lower the measured BMSi value. BMSi is generally decreased in individuals with prior low-energy trauma fractures compared with appropriate controls, and although measured at a site rich of cortical bone, low BMSi is associated with increased bone fragility at all relevant skeletal sites, vertebral, nonvertebral, and hip sites (4–7). Previous data thus suggest that tissue material properties of bone are altered in low-energy trauma fracture patients, and that BMSi measured at the tibia is associated with increased bone fragility at all relevant skeletal sites. In addition, BMSi is not related to BMD values or to microarchitectural parameters (4).

Experience has been accumulating with the use of this technique in research setting, and OsteoProbe® is approved for use in, eg, Europe and the United States (CE mark, FDA), where devices are used in the clinic as well (Europe, United States, and Australia), and, a standard operating procedure for IMI has been published to harmonize collection of data (8). As IMI is being increasingly used, the question arises whether this minimally invasive procedure is safe and accepted among patients. Previous data showed that IMI is well tolerated during and immediately after the procedure (9). Yet there is no data available about the longer-term safety and acceptability of this technique. Therefore, the aim of this study was to prospectively follow patients who underwent an IMI measurement for various reasons using the OsteoProbe® device at the Center for Bone Quality of the Leiden University Medical Center in order to evaluate the longer-term safety and acceptability of an IMI measurement. The

primary outcome was the 30-day complication rate after the measurement, and we hypothesized that this was low and without any major complications.

Patients and Methods

Study design

Prospective observational single-center study evaluating the safety and acceptability of an IMI measurement by questionnaires. The primary outcome was the 30-day complication rate after the measurement. The study was conducted in men and women attending the outpatient clinic of the Center for Bone Quality of the Leiden University Medical Center (LUMC) between September 2019 and December 2020. Inclusion period was prolonged due to the coronavirus disease 2019 (COVID-19) pandemic.

Patients

Included in the study were all consecutive patients aged ≥18 years who were scheduled for an IMI measurement by their treating physician due to various reasons and consented to participate in this study. These were mainly patients from the outpatient clinic of the Center for Bone Quality who were investigated for fragility fractures in the presence of normal BMD or osteopenia or patients without fractures and with osteoporosis in whom IMI served as a clinical diagnostic tool. A minority were patients with endocrine disorders from the outpatient clinic of the Endocrine Department with specific endocrine conditions that are associated with an increased fracture risk, eg, primary hyperparathyroidism and Cushing's syndrome. None of the participants scheduled for IMI were not able to undergo the measurement. Written informed consent was obtained from all individuals included in the study and the collection and analyses of the data has been approved.

Methods

Patients were asked to review the IMI procedure through a questionnaire at three time points by study personnel: at the outpatient clinic right after the procedure in form of a personal interview through the investigator and by telephone interviews at 1 week and 1 month after the procedure. The questionnaires (given as Supporting Information) were composed of safety-related questions and patients were asked to rate difficulty and pain intensity related to the procedure on visual analogue scales (VAS 1–10) with one being no difficulty or no pain (as shown in the Supporting Information). In addition, patients were asked during telephone interviews to send on a photograph of the indentation site if there was a visible hematoma or other skin

changes. Patients who reviewed the IMI procedure right after the measurement but could not be reached within the follow-up period were called at the end of the study period to be asked about presence of any complications of the procedure. In addition, a full medical history including fracture history, use of medication, and data on clinical risk factors for fractures were obtained from all patients.

IMI

In all patients, the IMI procedure was performed by three experienced operators using the handheld microindentation device OsteoProbe® (Active Life Scientific, Santa Barbara, CA, USA) on the midshaft of the tibia according to the standard operating procedure (8) and our previously published protocol (10). The time required for a measurement is approximately 10 min. In brief, the patient is placed in a position with the tibia in external rotation. In that position the flat surface of the medial tibia diaphysis is in a horizontal position where it can be assessed. The measurement site is set by the mean distance between the medial malleolus and the distal apex of the patella. After the correct position is chosen, the area is disinfected and the skin and periosteum are infiltrated with lidocaine 1%, usually between 5 and 10 mL. After this the test probe is gently inserted in the skin until the bone surface is reached (Fig. 1). The test probe is always perpendicular to the bone surface during measurements. Without pulling the tip out of the skin, a minimum of eight indentations are performed with each indentation 2 mm away from the previous indentation. The operator performs the indentations without visual confirmation of the results, and at

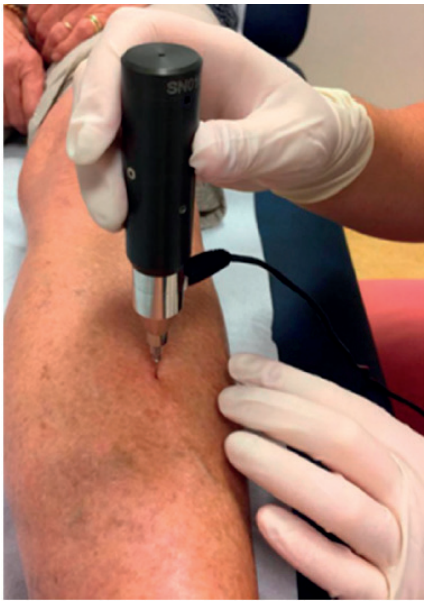

Fig. 1. Use of the OsteoProbe® on the midshaft of the tibia after application of a local anesthetic.

least five adequate indentations are required. Next, five additional indentations are performed on a polymethylmethacrylate (PMMA) calibration phantom. The outcome parameter BMSi is calculated by the computer software. Postprocedure only a small bandage is applied, but in the majority of cases there is only a small puncture hole visible without bleeding. There are no restrictions after the procedure. The Center for Bone Quality Leiden is a center of excellence for this technique and is one of three European training centers. In addition, all three OsteoProbe investigators who performed the procedure were provided training, including a didactic session describing subject selection and device usage as well as hands-on training on the use of the OsteoProbe®. At start of the study, operator 1 had IMI-experience of 1 year and 3 months, operator 2 had experience of 1 year and 7 months, and operator 3 who was the most experienced had been using IMI for 6 years and 4 months.

Statistical analyses

Normality of distribution of all values was checked by a Kolmogorov-Smirnov test and visually with histograms. Results are presented as mean±SD unless otherwise specified. Descriptive statistics were used to assess clinical characteristics, BMSi values, and data assembled through the questionnaires. The Kruskal-Wallis test was used to calculate interoperator differences in reported pain, and the Mann-Whitney U test was used to calculate differences in reported pain between two operators and between women and men. Statistical analyses were performed using IBM SPSS Statistics (IBM SPSS Statistics for Windows, Version 25.0; IBM Corp., Armonk, NY, USA) and graphs were constructed with GraphPad Prism (version 8.0; GraphPad Software Inc., La Jolla, CA, USA).

Results

All 106 eligible patients measured during the study period consented to participate in this study and were included in the baseline analysis. Of those, two patients were traveling and could not be reached for the 1-week follow-up, leaving 104 patients included in the 1-week follow-up analysis. Both patients were contacted in the weeks thereafter and thus included in the 1-month follow-up analysis (Fig. 2). Patients were contacted after a median of 10 days (interquartile range [IQR], 7–11 days) for 1-week and 31 days (IQR, 29–34 days) for 1-month follow-up analysis.

Characteristics of all 106 participants (71 women) are shown in Table 1. Median age at moment of measurement was 59 years (range, 20 to 86 years) and mean body mass index (BMI) was 26.7 ± 4.6 kg/m2. The mean BMSi value was 78.3 ± 7.1 and ranged from 59.5 to 99.8.

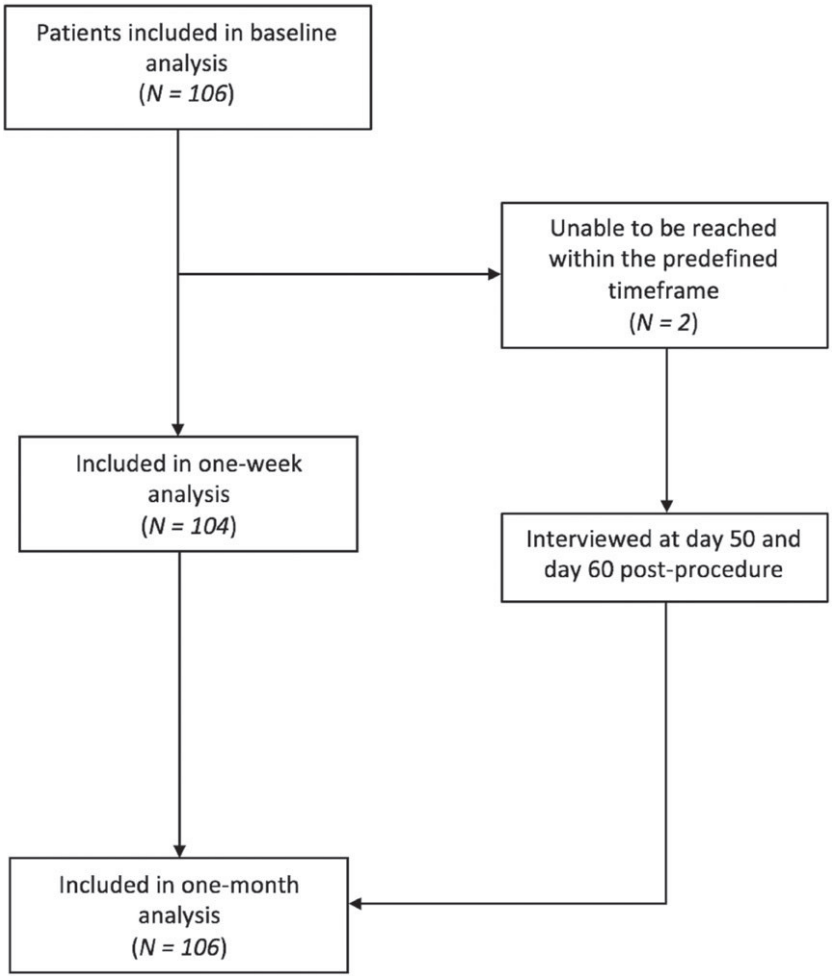

Fig. 2. Patient flowchart.

Adverse events

Table 2 summarizes the overall safety profile of IMI within our study. The primary outcome was the 30-day complication rate after the measurement. The overall event rate was 2.8%. Only three minor events were observed at 1-week follow-up: two patients experienced a very small hematoma at the indentation site, and one patient experienced a small bruise at the indentation site. One of the patients with a small hematoma used antiplatelet drugs at the moment of measurement due to stent placement after myocardial infarction. These minor events were all very mild in severity. No medical intervention was necessary nor did the patients worry, and all three events resolved by the 1-month follow-up. None of the patients experienced any adverse events between 1-week and 1-month follow-up.

Table 1. Baseline Characteristics

Characteristics	Patients (*n* = 106)
Age, years (range)	59 (20.0–86.0)
Gender (male/female)	35/71
BMI	26.7±4.6
Smoking, *n* (%)	17 (16)
Alcohol use, >3 IU/day, *n* (%)	9 (8.5)
History of fragility fracture, *n* (%)	26 (24.5)
BMSi (range)	78.3 ± 7.1 (59.5–99.8)

Values are expressed as mean±SD, except for age (median). Abbreviations: BMI = body mass index; BMSi = bone material strength index.

Infection

None of the 106 patients reported any signs compatible with local infection (redness, pus, or warmth at the site) at 1-week or at 1-month follow-up.

Medical care

Postprocedurally, none of the 106 patients had to seek medical care due to any reason related to the procedure nor did any of them have other safety-related problems or concerns related to the procedure.

Table 2. Summary of Adverse Event Rates

	Events	Patients (n = 106)	%
Adverse events			
All	3	3	2.8
Device related[a]	0	0	0.0
Procedure related[a]	3	3	2.8
Serious adverse events			
All	0	0	0.0
Device related[a]	0	0	0.0
Procedure related[a]	0	0	0.0
Adverse events by severity			
Mild	3	3	2.8
Moderate	0	0	0.0
Severe	0	0	0.0
Unknown	0	0	0.0
Death			
All	0	0	0.0

Adverse events: two patients experienced a small hematoma and one patient a small bruising at 1-week follow-up. Hematoma/bruising resolved in all three patients prior to 1-month follow-up. [a]Related defined as: Definite, Probable, Possible, and Unknown.

Experienced pain

Figure 3 presents the experienced pain rated according to the VAS (1–10) at the three moments of assessment. The vast majority of patients had no pain (VAS 1) at baseline, 1-week and 1-month follow-up (80.2%, 88.4%, and 94.3%, respectively). Twenty-five patients (23.6%) reported the application of a local anesthetic precedent to the measurement to be an unpleasant experience. At baseline visit immediately postprocedure, the pain experienced differed significantly between the group of patients measured by operator 1 (n = 40), operator 2 (n = 29), and operator 3 (n = 37). The median pain experienced according to the VAS was 1.0 (IQR 1.0–3.0; range, 1 to 8) for operator 1, 1.0 (IQR 1.0–1.0; range, 1 to 5) for operator 2, and 1.0 (IQR 1.0–1.0; range, 1 to 5) for operator 3, respectively (p = 0.007). The difference was significant between operator 1 and 2 (p = 0.02) and between operator 1 and 3 (p = 0.008), but there was no difference between operators 2 and 3 (p = 0.97), meaning that the most pain was observed in patients who were measured by the least experienced operator. There was no significant difference in reported pain between the three groups at 1-week (p = 0.49) or 1-month follow-up (p = 0.66). In addition, there was no difference in experienced pain between women (n = 71) and men (n = 35) at baseline (p = 0.05), 1-week (p = 0.06), nor 1-month follow-up (p = 0.12). Table 3 summarizes the results of the safety follow-up questionnaire immediately postprocedure, at 1 week and at 1 month following the procedure. All 106 patients had no difficulty with the procedure and would undergo the measurement again if needed. All but one patient found the measurement acceptable for other patients while one patient did not want to judge if it was for another person and thus did not answer this question.

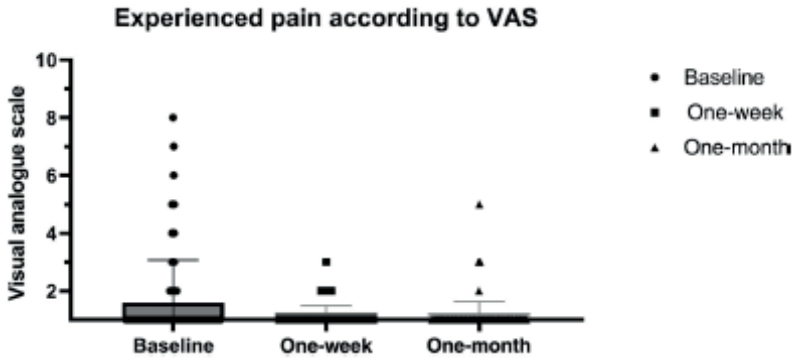

Fig. 3. Experienced pain according to the Visual Analogue Scale (VAS 1–10) at baseline, 1-week, and 1-month follow-up. Data are shown in box-whisker plots. Boxes indicate median and interquartile range. Bars indicate minimum and maximum values.

Table 3. Safety Follow-Up: Postprocedure Questions at Baseline, 1 Week, and 1 Month

Postprocedure questions	Baseline visit (n = 106)	One-week follow-up (n = 104)[a]	One-month follow-up (n = 106)
Have you experienced any safety related concerns or other problems related to the procedure? (yes/no)	0/106	0/104	0/106
Did you need to take pain relieving drugs for pain related to the procedure? (yes/no)		0/104	0/106
Are you concerned about bruising or bleeding around the measurement site? (yes/no)		3/101	0/106
Is there any sign of redness, pus, or warmth from the bone indentation site? (yes/no)		0/104	0/106
Have you sought any medical care due to the procedure? (yes/no)[b]			0/106

[a]Two patients could not be reached within the 1-week time period but were interviewed for the 1-month assessment.

[b]This question was only asked at 1-month follow-up.

Discussion

In this longitudinal observational study, we found the 30-day complication after a measurement to be very low with 2.8% and thus IMI to be a highly safe procedure. There were no safety-related concerns postprocedurally. In particular, no signs compatible with local infection were reported at all three moments of assessment, and all patients reported willingness to undergo the procedure again, and found the measurement acceptable for other patients.

Although IMI is a transcutaneous, microinvasive procedure, there were no serious adverse events neither immediately postmeasurement nor at 1-week or 1-month follow-up. In fact, only three minor adverse events were observed. These were a small bruise at the indentation site in one patient and a very small hematoma in another two patients, one of whom was using antiplatelet drugs. These were all mild in severity and were referred to as the measurement spot still being visible, and they all resolved by the 1-month follow up. These results complement and extend those by Rufus-Membere and colleagues (9), where no adverse events were found, although they report safety-results only immediately postprocedurally. Minor events such as bruising, which was only present at 1-week follow-up in our study, could have been missed in their report. Our results therefore add that IMI is safe in both the near-term and the longer-term. In particular, no signs compatible with local infection were reported at all three moments of assessment, an adverse event one would most likely fear due to the minimally invasive aspect of the procedure. Our results are also in accordance with the findings of a recent systematic review by our group including 38 publications, of which 14 reported that the IMI investigation using the

OsteoProbe® device was well tolerated and not associated with any major complications (4). According to the review, only one case of a mild local skin infection in a kidney transplant recipient that quickly resolved to oral antibiotic treatment has been reported to date. Another minor adverse event found in the literature review was a mild anaphylactic reaction to the local anesthetic used, which also resolved after medical treatment. Furthermore, an Investigational Device Exemption (IDE) clinical trial that focused on the safety of the procedure and was completed in 2020 only reported one mild adverse event, namely joint pain with a reported pain of 1 out of 10 on the Numeric Rating Pain Scale (11). No other adverse events have been reported so far.

Furthermore, the majority of the patients had no pain at all three moments of assessment. Actually, the only unpleasant aspect of the procedure was the application of local anesthetic before IMI, reported by only a minority of patients. Median pain perceived in our study was 1.0 on a VAS scale (1–10) with 1.0 indicating no pain. This finding is very similar to the findings of a population-based study from Australia by Rufus-Membere and colleagues (9). In a sample of 252 men with a mean age of 63 years, the pain experienced on a VAS scale (0–10) immediately post-IMI was also very low, with a mean of 0.4 ± 0.7. There, measurements were performed by one single trained operator. In contrast, in our study three trained operators performed the IMI measurements. Analyses by operator showed that pain experienced was lowest in the group of patients measured by the operator with the most experience. This highlights the importance of operator training and the regular use of IMI. Nevertheless, pain was very low in all three groups per operator, and the difference was not significant anymore when asking the patients at 1-week and 1-month follow-up. In addition, all patients would undergo the measurement again if needed, thus independent of the operator and pain experienced.

Patients found the measurement also acceptable for other patients. Although numbers are small, the tolerability of the procedure thus seems to be very high. To further improve patient satisfaction maybe the application of local anesthesia could be improved, since this was stated as an unpleasant aspect of the measurement by a minority of patients. Next to injecting the lidocaine very slowly, this could probably be done by adding bicarbonate to the local anesthetic solution, as suggested by a systematic review article, although experience of such a mixture with the use of IMI is missing (12).

Our study has strengths as well as limitations. One of the strengths of our study is the inclusion of women and men with a wide age range. It confirms the safety and acceptance of the procedure, and it is the first study to provide detailed follow-up information also among a female population and we found no significant differ-

ences in subjectively experienced pain after IMI between both genders. In addition, longer-term safety data up to 1 month were collected with no patient lost to follow-up. Both patients who were unable to be reached within the follow-up period were interviewed at the end of the study period. A limitation that has to be acknowledged is that telephone interviews might not assess all possible side effects of IMI when compared to face-to-face consultations. A recent systematic review including orthopedic and postoperative follow-up studies, however, suggests that telemedicine is a safe, valid and comparable method of consultation (13), although the review confers to a clinical, and not to a research setting. Another limitation of our study lies in a possible but inevitable bias concerning pain sensation, because patients with more unpleasant experiences in the hospital may report a higher pain-score or recall the IMI procedure to be a more painful experience. However, considering the median low pain-score in our study this seems not to be of concern.

In conclusion, our study shows that although minimally-invasive, IMI using the OsteoProbe® device at the midshaft of the tibia is a very safe and well-accepted procedure in a research setting. IMI offers useful information about the quality of the bone and is complementary to bone density measurements using DXA in the hands of an experienced operator. However, further studies are warranted to quantify the value of the device in predicting future fractures.

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SUMMARY AND GENERAL DISCUSSION

Chapter 8

SUMMARY OF THIS THESIS

Identifying the patient at increased risk for a fragility fracture remains challenging, especially in patients with secondary causes for osteoporosis, as BMD measurements using DXA have been shown to underestimate true fracture risk not only in secondary but also in primary osteoporosis (1-5). Over the past two decades, evidence has been accumulating for the need to evaluate bone strength beyond DXA BMD values in the assessment of true fracture risk. The Impact Microindentation technique has been demonstrated to be valuable in this respect by allowing the in vivo assessment of tissue-level material properties of bone, which significantly contribute to bone strength, in addition to the contribution of BMD.

Although several studies using the handheld OsteoProbe® device at the location of the mid-shaft of the tibia have demonstrated that measurements of BMSi are independent of measurements of BMD in the prediction of bone fragility, there are still questions to be addressed about the added value of its use in addition to DXA in the clinic, particularly in secondary osteoporosis, and of the general short- and longer–term safety of the technique.

The main findings of the studies included in this thesis are briefly summarized in this chapter.

PART I: INTRODUCTION

In Part I of this thesis, we set the scene for exploring the broader added value of IMI in the evaluation of bone strength in clinical practice by conducting a comprehensive systematic review of all published literature on the topic up to the year 2019. In **Chapter 2** we provide the results of data obtained from all 38 clinical studies using the IMI technique in vivo in humans up to this year, also addressing practical aspects of the technique. We also identified gaps and inconsistencies in the methodology and interpretation of data, mainly related to the lack of a standard operating procedure for the technique up to its publication in 2016 (6). Data from these 38 studies highlight the ability of IMI in identifying patients with increased bone fragility. This includes patients with primary osteoporosis with prevalent fragility fractures, especially those with osteopenia, most of whom are not currently being treated with bone-modifying agents under commonly adopted nationwide protocols. Findings from this systematic review consolidate the view that IMI may indeed represent a valuable tool for identifying patients with primary osteoporosis at increased fracture risk, particularly those with osteopenia, in whom DXA fails to adequately evaluate that risk. Although limited to three smaller studies, the review also suggests the ability of the technique in detecting changes in BMSi **i**n patients on bone-modifying therapies thus highlighting the potential role of IMI in monitoring patients with

elevated fracture risk. Results further underscore the value of IMI in patients with or at risk for secondary osteoporosis due to various systemic disorders including endocrine conditions such as diabetes mellitus or acromegaly. From safety data gathered for our systematic review, IMI is reported to be well tolerated during and immediately after the investigation, but there are so far no published data on the longer-term safety and acceptability of IMI measurements performed with the handheld OsteoProbe® device.

PART II: EVALUATION OF BONE FRAGILITY USING IMPACT MICROINDENTATION IN PRIMARY AND SECONDARY OSTEOPOROSIS

Part II of this thesis addresses the value of IMI in the assessment of bone strength not only in patients with low-energy trauma fractures, in the majority of whom BMSi values have been previously documented to be lower compared to non-fracture controls, but also in subjects with high-energy trauma fractures in whom this has never been previously investigated. In addition, this part explores the ability of IMI to detect longitudinal changes in BMSi in response to anti-osteoporotic treatment and its contribution to the evaluation of bone fragility in secondary osteoporosis due to endocrine disorders, specifically primary hyperparathyroidism and endogenous Cushing's syndrome (CS).

In Chapter 3, results of BMSi measured in forty patients with high-energy trauma fractures, who were attending our fracture liaison service (FLS) or the outpatient clinic of the Center for Bone Quality of the LUMC, were compared with those of gender- and age-matched patients with comparable fractures due to low-energy trauma also attending the FLS. Patients who had sustained one or more high-energy trauma fracture(s) were found to have significantly higher BMSi than their matched controls who sustained a low-energy trauma fracture. Data from this study also show that BMSi values did not differ between patients with osteopenia and those with osteoporosis within each trauma group. BMSi values of patients with high-energy trauma fractures were otherwise comparable to those previously reported in subjects without a fracture (7, 8). These data suggest that tissue-level properties of cortical bone are not impaired in patients with high-energy trauma fractures.

In Chapter 4, we address the issue whether changes in BMSi values are observed over time in patients receiving anti-osteoporotic treatments. We also assessed whether any observed changes in BMSi might provide additional information to that provided by changes in BMD measurements. To this effect, we assessed the longitudinal changes in BMSi and in BMD in treatment-naive patients with osteopenia or

osteoporosis with or without fractures who were treated with either antiresorptive agents in the form of bisphosphonates or Denosumab with vitamin D ± calcium supplements, or with only vitamin D ± calcium supplements (control group) following Dutch national guidelines for the management of osteoporosis. Our results show that treatment with bisphosphonates or denosumab for a mean period of 2 years, increases BMSi values in patients with low bone mass and increased risk of fracture, while they remain unchanged in the control group. The values of BMSi reached with antiresorptive treatment were comparable to the baseline values of the control group, as well as to those previously reported in patients with low bone mass without fractures (7, 8). The magnitude of BMSi changes depended on baseline values with larger increases observed in patients with the lower baseline values. Lumbar spine and total hip BMD values also increased in patients treated with bisphosphonates or denosumab, but the observed increases in BMD values were not associated with changes in BMSi values. Findings from this study thus suggest that IMI can capture BMD-independent, treatment-induced changes in BMSi, and may further be a useful addition to currently available tools for the assessment and follow-up of patients at increased fracture risk.

The assessment of BMSi using IMI is of particular interest in the evaluation of patients with secondary osteoporosis due to endocrine diseases, in which traditional measurements of BMD have been shown to underestimate fracture risk. In **Chapter 5**, we evaluated tissue-level properties of bone in 37 men and women with primary hyperparathyroidism (PHPT), and in 37 non-PHPT controls matched for gender, age and fragility fracture status. Patients and controls were studied at the outpatient clinic of the Department of Endocrinology of the LUMC which is a national expertise center for PHPT**.** In keeping with a preliminary report by Starr et al., published in abstract form, which demonstrated significantly lower BMSi values in 13 subjects with PHPT compared to 22 non-PHPT matched controls (9), our findings demonstrate significantly lower BMSi values in patients with PHPT compared to non-PHPT controls, independently of BMD values. Our data also show significantly lower BMSi values in patients with PHPT and prevalent fragility fractures compared to patients with PHPT without fragility fractures, and that BMSi values were inversely related to the severity of the hyperparathyroidism. These data indicate that tissue-level properties of bone are impaired in patients with PHPT, potentially contributing to the higher fracture risk observed in this population. This increased risk appears disproportionate to what would be anticipated based on BMD measurements alone and is likely to be attributable to impairments in material properties affecting both cortical and trabecular bone compartments.

In **Chapter 6,** we report the findings of a study on the value of BMSi in the assessment of fracture risk in endogenous Cushing's syndrome (CS), a markedly less

frequent endocrine disease than PHPT, although also associated with osteoporosis and fragility fractures as main clinical manifestations. In these patients, BMD has been observed to increase toward values within the normal range following successful treatment and remission of the disease, but fracture risk remains increased. At the LUMC, which is the coordinating center of the European Reference Network for Rare Endocrine conditions, and a European reference center for pituitary and adrenal diseases, we have a well-characterized cohort of patients with CS in remission after treatment. To further investigate the discrepancy between BMD data and fracture risk, we evaluated BMSi values in 60 patients with CS, a median of 6 years after achieving remission, and compared their data with 60 gender-, age- and BMD-matched controls. We found significantly lower BMSi values in patients with CS in remission than in controls, and BMSi values were also negatively correlated to BMI in patients with CS in remission compared to controls. This finding might reflect the length and severity of cortisol exposure and its deleterious effects on tissue-level properties of bone during active disease, which may persist and may be permanently impaired after complete CS remission is achieved. Strikingly, BMSi values of our CS cohort after long-term remission were comparably low as those measured in subjects treated with exogenous glucocorticoids, known to rapidly increase fracture risk, for more than 20 weeks for various underlying disorders in another study (10). These data highlight the necessity of a comprehensive evaluation of bone health in all patients with CS, both at the time of diagnosis and also after long-term remission.

PART III: EVALUATION OF THE SAFETY AND ACCEPTABILITY OF THE IMPACT MICROINDENTATION TECHNIQUE

IMI is being increasingly used in studies evaluating the contribution of tissue material properties to bone fragility in humans, and the OsteoProbe® has been approved for use in the clinic in Europe and the United States. Previous data show that IMI is well tolerated during and immediately after the procedure (11). However, it is important to establish whether this transcutaneous minimally invasive procedure is safe during and immediately after the procedure and whether it is well accepted by patients.

In **Chapter 7**, we address these issues by prospectively following patients with primary or secondary osteoporosis who participated in studies included in this thesis for 30 days after IMI measurements. The thirty-day post-IMI complication rate was found to be very low at 2.8%. No serious adverse events were observed neither immediately post-measurement, nor at the 1-week or at the 1-month follow-up time points. Only three minor adverse events were noted: one patient developed a small bruise at the indentation site, and two patients experienced very small hemato-

mas—one of whom was receiving antiplatelet therapy (Aspirin). These events were mild, described as a visible measurement spot, and had resolved by the 1-month follow-up time point. In none of the patients were clinical signs of local infection observed at any of the time points of assessment. From the acceptability point of view, all patients expressed their willingness to undergo the procedure again and rated the measurement acceptable for use in other patients. These data confirm that although minimally-invasive, IMI using the OsteoProbe® at the site of the midshaft of the tibia is a very safe and well-accepted procedure in the hands of an experienced operator, as is the case in our center.

Chapter 9

GENERAL DISCUSSION AND FUTURE PERSPECTIVES

An increasing body of evidence that has accumulated over the past two decades suggests a promising added value of Impact Microindentation using the OsteoProbe® in the evaluation of the contribution of bone tissue-level properties to bone strength. During this time span, it has also become clear that these features may not be captured by the sole measurement of BMD. Our Systematic Review evaluated data from all 38 published studies on the use of IMI, particularly with the use of the OsteoProbe®, up to the year 2019 (12). The review also highlighted the added value of BMSi in identifying fracture risks that were underestimated by the alone use of DXA BMD measurements. There have been only few additional publications on IMI since 2019. An important addition to the literature since then is the reporting of the results of a landmark Multicenter International study by Rufus-Membere et al (13), who set out to define reference interval data for BMSi in healthy adults by analysing *a posteriori* data collected from 479 healthy adult men and women aged 25 to 98 years, from seven research centers from Europe, Australia and the United States of America, including healthy subjects who participated in our IMI studies at the LUMC. Associations between BMSi, age, sex and areal BMD were examined. This study provided the so far missing healthy reference data values that can be used in the calculation of T- and Z-scores for BMSi and thus help confirm the ability of BMSi in predicting fracture risk beyond BMD. However, these data still need to be confirmed in larger prospective studies including populations from additional different geographic regions, as geographical differences are likely to influence BMSi values (12, 14). As discussed in our Systematic Review article, age-matched non-fractured women from Sweden had strikingly lower BMSi values compared with those from The Netherlands or Spain. These variations may also be reflected in differences in fracture risk and should thus be considered when establishing future national reference values, or when comparing data from studies conducted in different geographical areas. Optimal cut-off values to identify those at high fracture risk who might benefit from treatment also remain to be defined.

Importantly, evidence for a diagnostic association between BMSi and prevalent fractures should be distinguished from evidence for the prospective prediction of future fractures. The value of IMI in predicting future fractures also needs to be established. Almost all studies to date used a cross-sectional study design measuring BMSi in patients with prevalent low-energy trauma fractures, also from our group (7, 8, 15-18). These studies showed lower BMSi values in patients who sustained a fracture compared to non-fracture controls. Very few studies used a longitudinal study design such as ours measuring BMSi in treatment-naive patients receiving antiresorptive drugs or calcium and/or Vitamin D alone. These studies were not powered with a fracture endpoint. In the longitudinal study reported in Chapter 4 we also include anecdotal data of two patients in whom fragility fractures occurred during the period of observation. Neither of the two patients was treated with antiresorp-

tive agents, as per the nationally adopted treatment protocols. Both demonstrated a significant decline of 8.5% and 8.9% in their BMSi values at follow-up compared to pre-treatment baseline values. To date, there is only one recently published prospective study from a Swedish group, which investigates the association between baseline BMSi measurements and incident osteoporotic fractures in a population-based cohort of 647 older women with a narrow age range of 75 to 80 years (19). During a median follow-up time of 6 years, 151 of these women sustained an incident fracture. An unexpected significant association was found between a higher and thus better baseline BMSi and an increased risk for incident fractures, also after adjusting for various confounders, or when considering the competing risk of death. This finding is intriguing given that most cross-sectional studies including some of ours have reported lower BMSi in subjects with prevalent fractures (12). However, earlier cross-sectional studies performed by the same Swedish group investigating a smaller subsample of the same cohort also didn't reveal an association between BMSi and prevalent fractures (20, 21). The missing association between BMSi and fractures not only in their earlier studies but also in this prospective study might possibly be explained by the unspecified potential inclusion of incident fractures irrespective of trauma type. It is possible that the inclusion of subjects with high-energy trauma fractures who we have shown to have higher BMSi values than those with low-energy trauma fractures might have diluted the results. Furthermore, data from our systematic review included in this thesis suggest that the measurement of BMSi might not be of much added value in assessing bone fragility in the presence of severely reduced BMD, which is in itself highly predictive of future fractures. Older women included in the Swedish cohort studies actually show some of the lowest reported BMD results among all published IMI studies, which might also partly explain the lack of association between BMSi and incident fractures during the period of observation. On the other hand, findings from our systematic review suggest that tissue material properties, as measured by BMSi using Impact Microindentation, may become more relevant in patients with less severely affected BMD, where bone quality rather than bone quantity is likely to have a greater impact on fracture risk. This hypothesis is further supported by findings from the two studies conducted in this thesis in patients with primary hyperparathyroidism and in patients with Cushing's syndrome in permanent remission.

Notwithstanding, there is still an unmet need for larger-scale prospective trials to be conducted using standardised operating protocols, and including patients with larger age and BMD ranges, to further investigate the value of IMI in predicting future fractures. This is particularly relevant in patients diagnosed with osteopenia, given that more than 50% of fragility fractures occur in patients with osteopenia (22-26) and that most patients with T-scores in that range are currently not being of-

fered treatment with bone-modifying agents according to most nationwide adopted treatment protocols.

Another aspect that needs to be investigated is the value of IMI in predicting outcome of bone-modifying treatments. Our findings from this thesis suggest that in patients with a BMD- or fracture-determined increased fracture risk, treatment with bisphosphonates or denosumab induced BMD-independent increases in BMSi values. The magnitude of treatment-induced BMSi increases depended on pretreatment BMSi values, with the larger increases observed in patients with the lower baseline BMSi values. Similar results were reported in an early prospective longitudinal IMI study in 52 patients receiving glucocorticoids for various underlying disorders concurrently treated with prophylactic anti-osteoporotic medication or calcium/vitamin D alone (10). A further cross-sectional study found that BMSi values were significantly lower in patients treated with bisphosphonates who had incident fractures during treatment compared to patients who did not sustain fractures during treatment (27). However, there are to date no data available from prospective studies powered for fracture endpoints. The threshold at which BMSi values are associated with increased fracture risk thus remains an open question, as does the BMSi threshold achieved during treatment above which the likelihood of a new fracture is lowest. Determining this threshold will help establish, in a treat to target approach, when treatment may be safely discontinued.

Taken together, results of this thesis suggest that IMI may be a valuable and safe tool not only for assessing bone fragility but also for monitoring patients, particularly those with a potentially underestimated fracture risk. BMSi is not a measure of bone mass and no clear relationship between BMSi and BMD has been established. Results of this thesis thus strengthen the notion that measurements of BMSi may be a valuable addition to currently available tools for the assessment of fracture risk, rather than a replacement for individual patient assessments.

However, despite these promising results, some issues with the use of IMI remain to be addressed. On the practical side the IMI technique requires the skills of an experienced operator, and although increasing numbers of young doctors are being trained with its use, this is currently limited to a few expertise centers. There are currently no Clinical Practice Guidelines for the use of the OsteoProbe®. The ability of BMSi to predict future fractures and treatment outcomes, as well as its added value beyond established fracture risk assessment, still needs to be validated in prospective multicenter trials using standardized operating protocols and harmonized operator training before IMI can be recommended for routine clinical use. In selected clinical scenarios in which fracture risk appears to be underestimated by

BMD measurements alone, IMI may provide complementary, BMD-independent information when performed by an experienced operator.

Chapter 10

NEDERLANDSE SAMENVATTING

Toegevoegde Waarde van Impact Microindentatie bij de Evaluatie van Botfragiliteit in de Klinische Praktijk

Botfragiliteit leidend tot osteoporose en de daaruit resulterende botbreuken veroorzaken wereldwijd aanzienlijke morbiditeit, verhoogde mortaliteit en hoge gezondheidskosten. Hoewel deze aandoening vooral voorkomt bij mensen boven de vijftig jaar, kunnen ook jongere patiënten worden getroffen, met name bij secundaire osteoporose als gevolg van onderliggende ziekten of langdurig medicatiegebruik. Hoewel botmineraaldichtheid (BMD), gemeten met DXA, al lange tijd de standaard is voor de diagnose van osteoporose en de inschatting van fractuurrisico, weerspiegelt deze slechts één aspect van botsterkte. Botsterkte wordt daarnaast bepaald door botgeometrie en -microarchitectuur, materiaaleigenschappen van de botmatrix (zoals collageen en mineralisatie) en het evenwicht tussen botaanmaak (formatie) en -afbraak (resorptie). Veranderingen in een of meer van deze factoren kunnen de mechanische eigenschappen van het bot verminderen en het fractuurrisico verhogen.

Bij osteopenie, een toestand waarbij de botdichtheid lager is dan normaal maar nog niet laag genoeg om van osteoporose te spreken, zijn de botten al verzwakt zonder dat dit altijd duidelijk zichtbaar is op standaardmetingen. Met name bij osteopenie en bij secundaire vormen van osteoporose wordt het fractuurrisico daarom vaak onderschat wanneer uitsluitend BMD wordt gemeten, wat de noodzaak van aanvullende diagnostische methoden benadrukt.

Impact microindentatie (IMI) is een minimaal invasieve methode om de materiaaleigenschappen van het harde buitenste deel van het bot rechtstreeks in het lichaam te meten. Met het draagbare OsteoProbe®-apparaat wordt, na lokale verdoving, op het midden van het scheenbeen (tibia) de weerstand van het bot tegen een gestandaardiseerde kracht gemeten, gevolgd door metingen op een referentiefantoom van polymethylmethacrylaat (PMMA). De software berekent hieruit de Bone Material Strength index (BMSi), een maat voor bothardheid, waarbij lagere waarden wijzen op verminderde weerstand. BMSi is geassocieerd met botfragiliteit op verschillende skeletlocaties, waaronder de wervelkolom en heupen, en correleert niet met BMD maar wel met andere botmateriaalparameters, zoals de samenstelling van de organische matrix en minerale eigenschappen van het bot.

De IMI-techniek wordt inmiddels toegepast in klinieken in Europa, Australië en de Verenigde Staten en werd in 2021 goedgekeurd door de FDA. Referentiewaarden voor mannen en vrouwen zijn gepubliceerd op basis van een internationale studie. Ondanks deze vooruitgang blijven er vragen bestaan over de optimale toepassing van deze techniek in de dagelijkse klinische praktijk.

In **hoofdstuk 2** wordt een systematische review gepresenteerd van alle tot 2019 gepubliceerde studies over IMI bij mensen. In totaal werden 38 klinische studies geïncludeerd. De review laat zien dat IMI in staat is patiënten met verhoogd fractuurrisico te identificeren, vooral degenen met osteopenie die door DXA mogelijk niet worden herkend. Daarnaast werden hiaten en inconsistenties in methodologie en interpretatie van de data geïdentificeerd, voornamelijk vanwege het ontbreken van een gestandaardiseerde procedure vóór 2016. Beperkingen van de beschikbare data zijn onder meer kleine studiepopulaties en beperkte informatie over langetermijnveiligheid, hoewel de techniek goed werd verdragen tijdens en kort na de metingen.

In **hoofdstuk 3** wordt onderzocht of BMSi verschilt tussen patiënten met hoog-energie en laag-energie fracturen. In 40 patiënten met hoog-energie fracturen werden BMSi-waarden vergeleken met leeftijd- en seksegematchte patiënten met laag-energie fracturen. De resultaten toonden aan dat patiënten met hoog-energie fracturen significant hogere BMSi-waarden hadden, terwijl er binnen elke groep geen verschil in BMSi was tussen patiënten met osteopenie en patiënten met osteoporose. Dit suggereert dat de weefselniveau-eigenschappen van corticaal bot niet zijn aangetast bij patiënten met hoog-energie fracturen.

In **hoofdstuk 4** wordt de invloed van antiresorptieve behandeling op BMSi onderzocht. Behandelings-naïeve patiënten met lage botmassa kregen bisfosfonaten of denosumab gedurende gemiddeld twee jaar, terwijl een controlegroep alleen calcium en vitamine D kreeg. Bij patiënten die antiresorptieve therapie kregen, nam BMSi toe, onafhankelijk van veranderingen in BMD, terwijl BMSi in de controlegroep ongewijzigd bleef. De grootste BMSi-toename werd waargenomen bij patiënten met de laagste baseline-waarden. Dit toont aan dat IMI behandelingsgerelateerde veranderingen in botkwaliteit kan detecteren die onafhankelijk zijn van veranderingen in BMD.

De beoordeling van BMSi met behulp van IMI is vooral van belang bij de evaluatie van patiënten met secundaire osteoporose door endocriene ziekten (hormonale aandoeningen), waarbij traditionele metingen van botdichtheid (BMD) het fractuurrisico vaak onderschatten. In **hoofdstuk 5** werd BMSi onderzocht bij patiënten met primaire hyperparathyreoïdie (PHPT), een hormonale aandoening waarbij de bijschildklieren te veel parathyroïdhormoon produceren, wat leidt tot botafbraak. De 37 patiënten met PHPT hadden significant lagere BMSi-waarden dan 37 niet-PHPT-controles, onafhankelijk van BMD. BMSi correleerde negatief met de ernst van de ziekte en het voorkomen van fragiliteitsfracturen. Deze resultaten suggereren dat verminderde materiaaleigenschappen van het bot bijdragen aan het verhoogde fractuurrisico bij PHPT.

In **hoofdstuk 6** beschrijven wij een studie bij patiënten met het syndroom van Cushing, een zeldzame ziekte waarbij het lichaam langdurig wordt blootgesteld aan te hoge spiegels van het stresshormoon cortisol, en het risico op botbreuken verhoogd is. Ook na succesvolle behandeling en genezing blijft het risico op botbreuken verhoogd, zelfs wanneer de botdichtheid weer normaal lijkt. Wij hebben de botkwaliteit onderzocht bij 60 patiënten die gemiddeld 6 jaar genezen waren, en vergeleken met 60 leeftijd-, geslacht- en BMD-gematchte gezonde personen. BMSi bleef duidelijk lager bij de patiënten, ondanks genormaliseerde BMD, en correleerde negatief met BMI. Dit wijst op blijvende schade aan botmateriaal door eerdere overmatige cortisolblootstelling en onderstreept het belang van uitgebreide evaluatie van botgezondheid bij patiënten met het syndroom van Cushing, zowel tijdens actieve ziekte als na remissie.

In **hoofdstuk 7** wordt de veiligheid en acceptatie van IMI onderzocht. Patiënten werden 30 dagen gevolgd na de procedure. Het complicatiepercentage was zeer laag (2,8%) en alle bijwerkingen waren mild en tijdelijk. Patiënten gaven aan bereid te zijn de procedure opnieuw te ondergaan, wat bevestigt dat IMI een veilige, goed geaccepteerde techniek is in de klinische praktijk.

Toekomstperspectieven

Samenvattend ondersteunt dit proefschrift het groeiende bewijs dat Impact Microindentation een waardevolle en veilige techniek is voor het beoordelen van botfragiliteit in vivo. Vooral bij patiënten bij wie het risico op botbreuken alleen door het meten van BMD mogelijk wordt onderschat, kan deze meting extra waardevolle informatie geven. De BMSi meet niet de hoeveelheid bot, en hangt niet rechtstreeks samen met de botdichtheid. Dit betekent dat beide metingen verschillende aspecten van de botgezondheid laten zien. De BMSi-meting kan daarom een belangrijke aanvulling zijn op de bestaande onderzoeken, maar vervangt deze niet.

Ondanks deze veelbelovende resultaten blijven er verschillende uitdagingen bestaan voordat IMI in de routineklinische praktijk kan worden geïntegreerd. Grootschalige prospectieve studies zijn nodig om leeftijds-, geslachts- en geografiespecifieke referentiewaarden te definiëren, klinisch relevante drempelwaarden voor BMSi vast te stellen en de voorspellende waarde voor toekomstige fracturen en behandelresultaten te bepalen. Gestandaardiseerde werkinstructies en training van de gebruikers zijn essentieel, evenals verdere validatie in multicenterstudies. Het is belangrijk te benadrukken dat IMI geen vervanging is voor BMD, maar eerder een aanvullend hulpmiddel dat extra, BMD-onafhankelijke informatie over botkwaliteit biedt en mogelijk patiënten met een hoog fractuurrisico identificeert die anders niet gedetecteerd zouden worden.

APPENDICES

Portfolio

Curriculum Vitae

Acknowledgements

Portfolio

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Curriculum vitae

Manuela Schoeb was born on 14 November 1984 in Niederuzwil, Switzerland. After graduating from high school in Wattwil, Switzerland, she studied Medicine at the University of Zurich, obtaining her Swiss Federal Medical Diploma in 2010 and her Doctor of Medicine (MD) degree in 2011.

Between 2011 and 2017, she completed her postgraduate training in General Internal Medicine at Kantonsspital St. Gallen and Spital Flawil, Switzerland. During this period, she also worked as an attending physician in Internal Medicine and at the Obesity Center of Kantonsspital St. Gallen. She obtained the Swiss Board Certification in General Internal Medicine in 2015 and the FMH specialist title in 2017.

From 2018 to 2020, she conducted the clinical and translational research that forms the basis of this thesis as a Clinical and Research Fellow in the Division of Endocrinology and the Center for Bone Quality, Department of Medicine, Leiden University Medical Center (LUMC), the Netherlands, under the supervision of Prof. dr. N.M. Appelman-Dijkstra and Dr. N.A.T. Hamdy. She was awarded the competitive European Calcified Tissue Society (ECTS) Clinical Research Fellowship and the European Union of Medical Specialists (UEMS) Exchange in Endocrinology Expertise (EEE) Grant, which supported the research presented in this thesis.

After returning to Switzerland in 2020, she completed her specialist training in the Division of Endocrinology, Diabetology, Osteology and Metabolic Diseases at HOCH Kantonsspital St. Gallen, obtaining her FMH specialist title in Endocrinology/Diabetology in 2023. Since 2023, she has been an attending endocrinologist in the Division of Endocrinology, Diabetology, Osteology and Metabolic Diseases at HOCH Kantonsspital St. Gallen, with a clinical and academic focus on osteoporosis and rare bone diseases. She currently leads the Center for Rare Bone Diseases of Eastern Switzerland and the Fracture Liaison Service at HOCH Kantonsspital St. Gallen.

She married Nicola Frei in 2020, and together they have a daughter.

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