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Diagnostic strategies in patients with high fracture risk

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DIAGNOSTIC STRATEGIES IN PATIENTS WITH HIGH FRACTURE RISK

FRANK MALGO



DIAGNOSTIC STRATEGIES IN PATIENTS WITH HIGH FRACTURE RISK

FRANK MALGO

The studies described in this thesis were performed at the Division of Endocrinology and the Center for Bone Quality, Department of Medicine of the Leiden University Medical Center, Leiden, the Netherlands.

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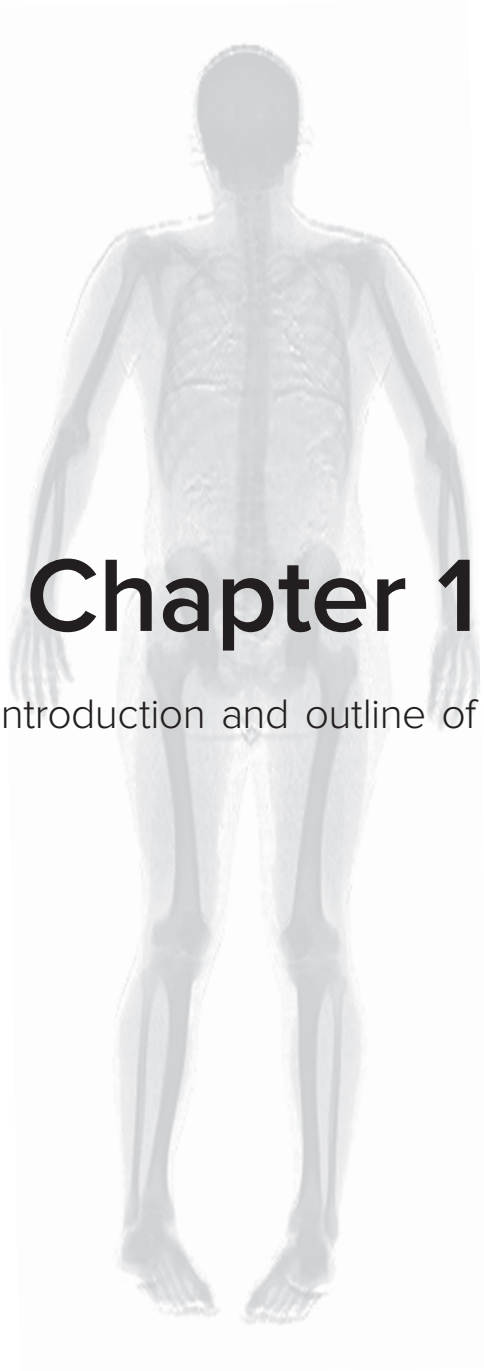
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Chapter 1

General introduction and outline of the thesis

GENERAL INTRODUCTION

Osteoporosis-related fractures represent a major health care problem demanding immediate attention from the physician. Osteoporosis is defined as a chronic disease characterized by low bone mass and a deterioration in bone microarchitecture and material properties of bone leading to decreased bone strength and increased risk of fracture (1). A fracture is the single most important clinical consequence of osteoporosis, the majority of which occur in postmenopausal women and old age. It is estimated that 3.5 million fractures occur each year in Europe (2). In the Netherlands, there are more than 800,000 women and men diagnosed with osteoporosis, with an estimated number of 49,000 fractures in women and 27,000 in men likely to occur each year (3). It is thus estimated that one in three women and one in five men aged 50 years or older will sustain a fracture in their lifetime. All fractures have been shown to be associated with increased morbidity and mortality and with a decrease in quality of life, all reflected in personal, societal and economic burdens (2, 4, 5).

And yet, despite accumulating evidence for the detrimental consequences of fractures, the number of patients who do not receive adequate attention after a recently sustained fracture remains alarming (6, 7). This issue has been clearly recognized by the International Osteoporosis Foundation leading to the launch of the global initiative of “Capture the Fracture” to increase awareness and attention for post-fracture care (8). This post-fracture care includes a number of facets ideally bundled in a Fracture Liaison Service (FLS), which consist primarily of a multidisciplinary care trajectory offering screening for osteoporosis using Dual Energy X-ray Absorptiometry (DXA), and for possible underlying secondary factors for increased fracture risk in the form of a panel of laboratory investigations in patients at high risk for fractures. Establishment of an FLS has clearly been shown to be associated with a decrease in the incidence of new fractures and in mortality (5). This approach has thus proven to be a successful formula in the management of osteoporosis by providing secondary fracture prevention in patients with a recent fracture in a cost-effective approach (9, 10).

In daily clinical practice, Bone Mineral Density (BMD) measurements using DXA remain the corner stone for the diagnosis of osteoporosis. BMD is measured at two main representative sites of the skeleton: the lumbar spine (L1-L4) and the femoral neck. T-scores are calculated using country-specific reference values obtained in groups of healthy young female subjects. The diagnosis of a normal BMD (T-score ≥ -1.0 SD), osteopenia (T-score > -2.5 and ≤ -1.0 SD) or osteoporosis (T-score ≤ -2.5 SD) is based on established World Health Organization criteria (11, 12). BMD has been shown to be a strong predictor for fractures, and the combination of BMD and age further

strengthens the predictive value of BMD for fractures (13, 14). Although the incidence of fractures increases with age, the majority of fractures in elderly patients has been shown to occur in the presence of osteopenia (13, 15).

There is increasing evidence that factors other than BMD determine bone fragility and thus fracture risk. These underlying secondary risk factors for fractures should be taken into account in the assessment of an individual's fracture risk. Several studies have shown that these other secondary factors for increased bone fragility are common in patients with osteoporosis (16–18), but evidence is also accumulating for a high prevalence of these factors in patients with osteopenia (16, 17). Secondary factors for fractures are numerous and may be identified by a detailed medical history including smoking and drug use particularly glucocorticoids, and evaluated and confirmed using appropriate laboratory investigations including thyroid and gonadal function, which may be expanded to include less common causes potentially contributing to bone fragility. It is important to identify these factors as a number may be reversible. Evaluation of secondary factors for fracture risk is included in the FRAX algorithm, a computer-based algorithm, accessible online (<http://www.sheffield.ac.uk/FRAX/index.aspx>), that makes use of the presence or absence of independent risk factors to generate a 10-year probability for a major osteoporotic fracture (clinical spine, hip, distal radius and humerus) and for a hip fracture (19, 20). The model input includes an individual's age, gender and BMI, and information on the presence or absence of the following risk factors: prior fracture, parental history of hip fracture, ever use of glucocorticoids, current smoking, excessive use of alcohol, rheumatoid arthritis and other secondary causes for osteoporosis (Table 1). Fracture risk prediction has been shown to be improved by including data on femoral neck BMD in the calculation although FRAX can also provide adequate fracture risk prediction without the inclusion of femoral neck BMD (21). A number of significant limitations has been identified, however, with the use of FRAX. There is thus no weight given for a likely dose response effect of glucocorticoid use, of smoking and other factors in the algorithm. In addition, FRAX does not take into account a number of risk factors significantly associated with fracture risk such as falls and frailty, biochemical markers of bone turnover and the presence of morphometric vertebral fractures (19).

The presence of one or more vertebral fractures has been thus shown to represent a strong independent predictor for future vertebral and for non-vertebral fractures (22, 23), and vertebral fractures have been shown to be associated with increased morbidity and mortality (24, 25). Assessment of vertebral fractures should therefore be incorporated in the evaluation of all patients with osteoporosis. Conventional radiographs of the spine remain the gold standard for screening for the presence of vertebral fractures. However, Vertebral Fracture Assessment (VFA) is advocated as a surrogate

Table 1. FRAX clinical risk factors

Patient characteristics:	
Age	... years
Sex	male/female
Weight	... kg
Height	... cm
Clinical risk factors:	
Previous fracture	yes/no
Parental hip fracture	yes/no
Current smoking	yes/no
Glucocorticoids	yes/no
Rheumatoid arthritis	yes/no
Secondary osteoporosis*	yes/no
Alcohol 3 or more U/day	yes/no
Bone Mineral Density:	
Femoral neck	...g/cm ²

kg kilograms; cm centimetres; U unit; g/cm² grams per square centimetres.

* Disorders strongly related with osteoporosis; type I diabetes mellitus, osteogenesis imperfecta, untreated long-standing hyperthyroidism, hypogonadism or premature menopause (< 45 years), chronic malnutrition, malabsorption and chronic liver disease.

screening method for the presence of vertebral fractures and has been increasingly used in clinical practice although its value is as yet to be formally validated (26). VFA is a technique available on the DXA machine using a software programme, which allows the patient-friendly evaluation of the presence of vertebral fractures in the same session as a BMD measurement. Lower radiation exposure, better time-management and possible lowers costs are considered to be potential benefits of using VFA compared with conventional spine radiographs (27, 28).

Over the past two decades, there has been accumulating evidence for a contributory role of factors other than BMD, such as bone structure, bone composition and material properties of bone to bone strength, although these factors have been so far difficult to assess. An increasingly used method for the evaluation of disturbances in bone architecture in clinical trials is High Resolution peripheral Quantitative Computed Tomography (HRpQCT), a non-invasive 3-dimensional imaging technique (29). This method can distinguish trabecular from cortical bone and is used to assess volumetric BMD at the distal radius and at the tibia. A number of studies, all conducted in postmenopausal women with osteoporosis, showed a relationship between poor bone microarchitecture and fracture risk (29–31). These studies also demonstrated that postmenopausal women with osteopenia and fractures had worse microarchitecture

than women without fractures (29, 32). A further study, also conducted in postmenopausal women with osteopenia, demonstrated a relationship between cortical porosity and fractures of the distal radius (33).

Another tool used to assess structural characteristics of bone is the Trabecular Bone Score (TBS), a non-invasive technique that uses DXA-generated lumbar spine images (34–36), to produce 3-dimensional microarchitectural parameters of bone (37, 38). TBS has been found to be predictive of fracture risk in several prospective studies, also showing improvement of the predictive value of TBS when combined with BMD data (39–42).

Despite the promising advances made possible with the use of these novel technologies, it remains difficult to assess material properties of bone. The gold standard for the assessment of these properties have so far been the compression test and the three-point bending test, but these are *ex vivo* methods undertaken on transiliac bone biopsy material and not otherwise applicable for use *in vivo* in the clinic. Reference Point Indentation is a relatively new microindentation technique that assesses bone's resistance to fracture in a minimally invasive manner (43, 44). The first clinical studies using cyclic microindentation (44), an earlier version of the technique, and impact microindentation (45, 46), the most recently improved version of the technique that is now increasingly used in clinical studies, have provided interesting insights into the material properties of bone, which are further discussed in **Chapter 5**. In short, impact microindentation makes use of a single impact device called the OsteoProbe, to indent the bone surface of the tibia, allowing the evaluation of material properties of bone at the cortical level. The distance covered by the test probe after impact is the Indentation Distance Increase (IDI), which is used to calculate the outcome parameter: Bone Material Strength index (BMSi) defined as the ratio of the harmonic mean of the IDI from a polymethylmethacrylate calibration phantom, to the mean IDI from the bone surface of the tibia (45). Over the past few years, this exciting field of investigation of bone material properties at the tissue level has gained increased momentum, particularly with increased evidence about the ability of this tool to capture aspects of bone fragility which are independent of BMD (47–51)(**this thesis**). Despite further technical improvements of the RPI device, a number of questions still remain to be answered before the use of this tool could be widely advocated in the routine management of patients with high fracture risk with or without osteoporosis.

OUTLINE OF THE THESIS

The first part of this thesis focuses on different aspects of the screening programme included in the Leiden University Medical Center's FLS. **Chapter 2** describes the prevalence of underlying secondary factors for increased bone fragility in patients who have recently sustained a fracture. In **Chapter 3**, we compared low-radiation VFA with conventional radiography of the spine in the identification of vertebral fractures. We were interested in the performance of VFA to identify vertebral fractures as this tool is being increasingly implemented in the clinic. Laboratory investigations are also part of the screening programme in our FLS to assess the presence of underlying secondary factors for increased bone fragility but bone turnover markers (BTMs) can also be easily measured from serum collected in the screening visit. **Chapter 4** reports on the predictive value of BTMs for treatment outcome after starting treatment with antiresorptive drugs.

Over a decade ago, Reference Point Indentation (RPI) was introduced as a new method for the *in vivo* evaluation of tissue-level material properties of bone. Impact microindentation (IMI) is the RPI technique currently used in humans *in vivo*, **Chapter 5** provides a detailed and up-to-date review on all published *in vivo* clinical studies using the IMI technique in humans. In **Chapter 6**, we assessed the relationship between the IMI technique and prevalent fragility fractures in patients with low bone mass. We further expanded studies in this cohort in **Chapter 7**, as we were interested in the relationship between IMI and different types of fragility fractures, especially vertebral fractures. It has been shown that patients with acromegaly have an increased risk for vertebral fractures in the presence of relatively normal BMD values. In **Chapter 8**, we evaluated Bone Material Strength index (BMSi) values as measured by IMI in patients with acromegaly compared to control patients. We also compared BMSi values in patients with acromegaly, with or without vertebral fractures. Although multiple studies have been published on IMI since its introduction, a number of questions remains unanswered, including the number of measurements necessary to calculate BMSi, the identification of correctly obtained measurements, insights on the variability of these measurements, and the preferred measurement site. In **Chapter 9**, we address these questions by using IMI to evaluate patients with Paget's disease of bone localized in the tibia compared to patients without tibia pathology.

Chapter 10 provides a discussion of the findings of this thesis.

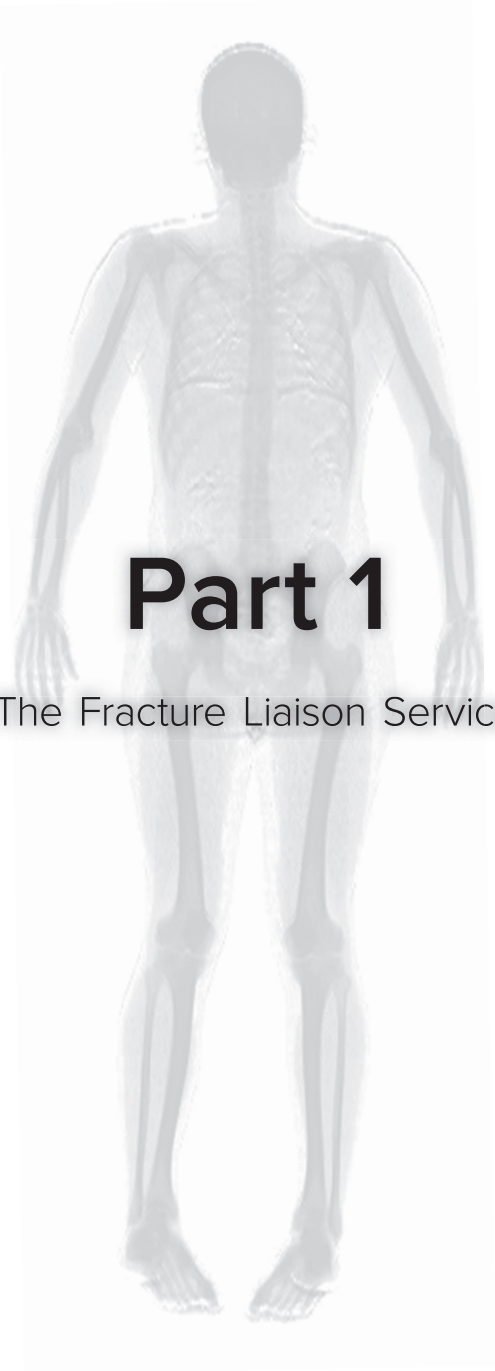
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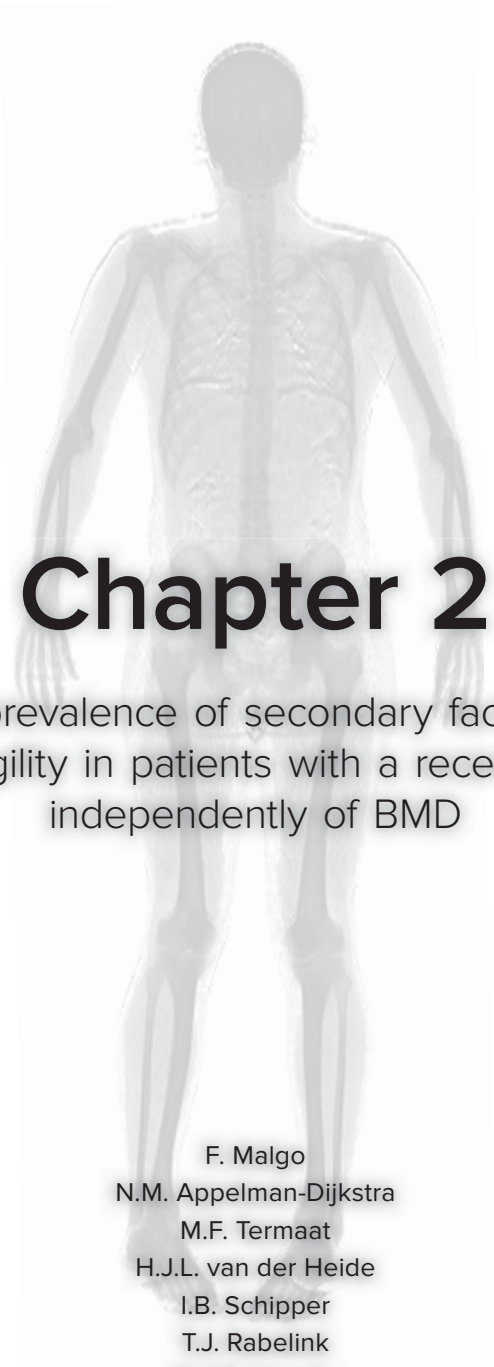
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Part 1

The Fracture Liaison Service



Chapter 2

High prevalence of secondary factors for
bone fragility in patients with a recent fracture
independently of BMD

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ABSTRACT

Summary:

In this study we demonstrate a high prevalence of secondary factors in patients with a recent fracture independently of BMD. Our results suggest that patients with a recent fracture should be screened for secondary factors for bone fragility regardless of BMD values.

Introduction:

Secondary factors for bone fragility are common in patients with osteoporosis who have sustained a fracture. The majority of fragility fractures occurs, however, in patients with osteopenia and it is not known whether secondary factors may contribute to fracture risk in these patients or in those with normal BMD.

Methods:

Prospective cohort study evaluating the prevalence of secondary factors for bone fragility in consecutive patients referred to our Fracture Liaison Service from June 2012 to June 2014 after a recent fracture.

Results:

709 patients were included, 201 (28%) with osteoporosis, 391 (55%) with osteopenia and 117 (17%) with normal BMD. Mean age was 66.0 ± 9.8 years, 504 (73%) were women and 390 (57%) had one or more underlying secondary factor. Evaluation of clinical risk factors using FRAX identified 38% of patients with ≥ 1 secondary factor including smoking (18%), excessive alcohol use (12%), glucocorticoid use (12%) and rheumatoid arthritis (3%). Laboratory investigations revealed chronic kidney disease in 13%, monoclonal gammopathy also in 13%, and primary or secondary hyperparathyroidism in 1% and 6%, respectively. Secondary factors for bone fragility were equally prevalent in patients with osteoporosis, osteopenia, or normal BMD.

Conclusions:

Our findings demonstrate a high prevalence of secondary factors for bone fragility in patients who have sustained a recent fracture, independently of BMD. The significant number of documented factors, which were treatable suggest that patients who sustained a fracture should be screened for secondary factors for bone fragility regardless of BMD values to optimize secondary fracture prevention.

INTRODUCTION

Osteoporotic fragility fractures are associated with increased morbidity and mortality and growing personal, societal and economic burdens [1–4]. The presence of a fragility fracture has also been shown to significantly increase the risk of future fractures [5, 6]. Over the past decade, Fracture Liaison Services (FLS) have been globally implemented to improve the identification and treatment of patients at risk for a new fracture in a cost-effective approach [7, 8]. Patients who sustain a fracture and who have osteoporosis are offered treatment with bone-modifying agents, but a significant number of patients who sustain a fracture have Bone Mineral Density (BMD) in the osteopenia range [9,10] and in the Netherlands, these are not generally screened for secondary factors for bone fragility and are not routinely offered anti-osteoporosis treatment [11, 12].

Secondary factors for bone fragility are common in patients with osteoporosis and a fragility fracture [13–15], but data on the prevalence of these factors in patients with osteopenia or normal BMD and a fragility fracture are scarce [13, 15]. Postmenopausal women with osteopenia and fragility fractures have been shown to have poor bone microarchitecture and altered material properties of bone, which may also be influenced by secondary factors for bone fragility [16–19]. To assess the potential contribution of secondary factors for bone fragility to fracture risk, we set out to evaluate the prevalence of these factors in a cohort of patients who had recently sustained a fracture and who were referred to the FLS for further investigation and management.

PATIENTS AND METHODS

Study design

Prospective cohort study in which all patients ≥ 50 years with a recent fracture, who were referred to the FLS of the Leiden University Medical Center from June 2012 to June 2014 were screened for secondary factors for bone fragility.

Patients

Patients were informed of their referral to the FLS during their follow-up visit for primary fracture care at the Outpatient Clinics of the Departments of Traumatology or Orthopaedic Surgery. Excluded from the study were patients with an isolated fracture of the skull, hands or feet, patients with pathological fractures or those with fractures resulting from failure of prosthesis. Patients who had undergone screening for osteoporosis in another hospital were also not included in the study. Patients with impaired cognitive function or poor general condition were also excluded from the study; Fig. 1.

Study parameters:

The following data were collected in all patients: age, gender, height and weight (from which Body Mass Index (BMI) was calculated), a full medical history including detailed fracture history, family history of osteoporosis, dietary calcium intake, age at menopause (early menopause was defined as menopause at age ≤ 45 years), parental history of hip fracture, alcohol use, corticosteroid use, smoking, current use of medication, including vitamin D supplementation, past or present use of hormone replacement therapy and past or present use of bone modifying agents. The 10-year probability for a major osteoporotic fracture and for a hip fracture were calculated using the FRAX algorithm using reference values for the Dutch population [20]. The 10-year fracture probabilities were calculated with and without inclusion of values for femoral neck BMD.

Bone Mineral Density

Bone Mineral Density was measured at the lumbar spine (L1-L4), and at the left and right femoral neck by Dual-energy X-ray Absorptiometry (DXA) using Hologic QDR Discovery A (Hologic, Bedford, MA, USA). T-scores were calculated using NHANES III reference values compatible with reference values for the Dutch population. The World Health Organization criteria were used to define osteoporosis, osteopenia and normal BMD. Average values of right and left femoral neck BMD were used in the analysis of data, except when both sides could not be measured due to the presence of a prosthesis.

Laboratory investigations

Serum was measured for calcium, albumin, inorganic phosphate, alkaline phosphatase, potassium, sodium, ureum, creatinine, TSH, PTH, 25-OH vitamin D and P1NP. Vitamin D deficiency was defined as serum levels of 25-OH vitamin D < 50 nmol/L. Renal function was assessed by calculating the estimated glomerular filtration rate (eGFR) using the modification of diet in renal disease (MDRD) formula. Stages of chronic kidney disease were defined as I to V according to the classification of the National Kidney Foundation KDOQI [21]. Primary hyperparathyroidism was diagnosed by hypercalcaemia (albumin corrected calcium > 2.55 mmol/L) in the presence of an inappropriately normal or elevated PTH level (PTH > 8.0 pmol/L), in the absence of thiazide use. Hypothyroidism was diagnosed by a repeated TSH level < 0.300 mU/L in the presence of a free T4 (fT4) level > 22.0 pmol/L and subclinical hyperthyroidism by a TSH level < 0.300 mU/L in the presence of a fT4 level between 10.0–24.0 pmol/L, without use of interfering medication. Hypogonadism was screened for in men < 70 years of age and was diagnosed by a total testosterone level of < 8 nmol/L in a morning sample. Screening for a monoclonal gammopathy using immunofixation was undertaken at the

discretion of the treating physician, mostly in case of unexpected osteoporosis and/or multiple fractures, also in the presence of osteopenia. Monoclonal Gammopathy of Undetermined Significance (MGUS) was defined by the presence of M-protein in serum at a concentration of up to 10g/L, with no signs of organ damage in the form of anemia, hypercalcemia, kidney insufficiency or bone lesions [22].

Statistical analysis

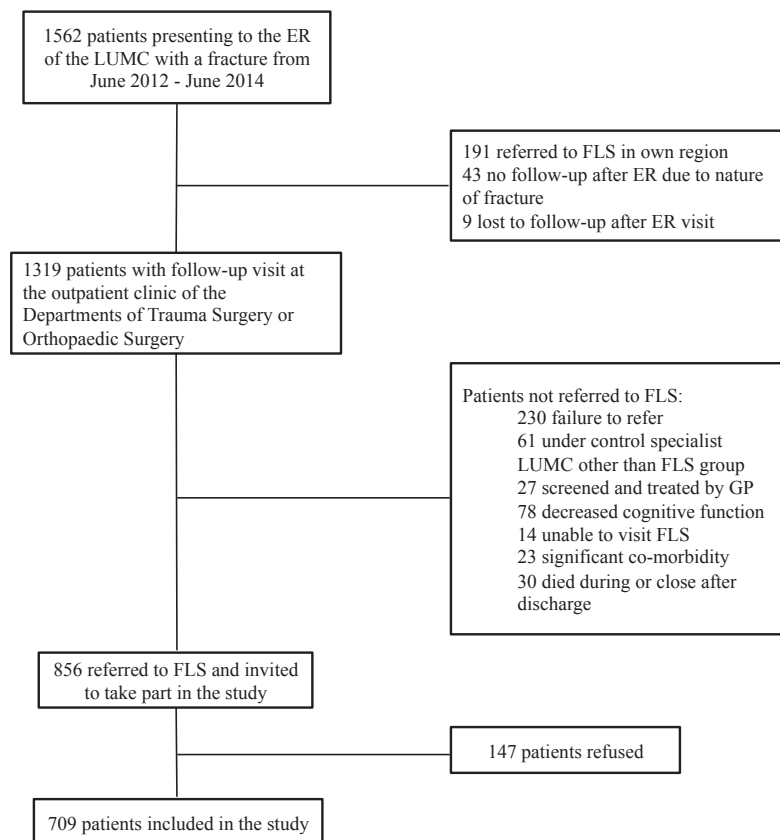
All analyses were performed using the SPSS software for Windows (Version 20.0; SPSS Inc., Chicago, IL, USA). Between group differences in baseline characteristics were assessed using ANOVA, a Chi-square test or a Kruskal Wallis test. The prevalence of secondary factors for bone fragility according to the recently sustained fracture type was assessed using a Chi-square test. Pearson correlation coefficients were used to express correlations between the 10-year FRAX probability calculated with and without femoral neck BMD values (after logarithmic transformation), lumbar spine and mean femoral neck BMD, and the number of secondary factors for bone fragility. Partial correlation coefficients were calculated to assess the correlation between number of secondary factors and lumbar spine and mean femoral neck BMD after adjusting for age, gender and BMI. Multivariate logistic regression analysis was used to assess the contribution of BMD: normal, osteopenia and osteoporosis (variable) to the prevalence of secondary factors for bone fragility (outcome), adjusted for age, gender and BMI.

Differences were considered to be significant at $p < 0.05$.

RESULTS

In the 2-year study period, 1562 patients presented to the Emergency Room of the Leiden University Medical Center with a recent fracture. 706 patients were not referred to the Fracture Liaison Service for a variety of reasons detailed in Fig. 1. Compared to this group, the 856 patients who were referred were younger (68.6 ± 11.2 years vs. 72.2 ± 12.9 years, $p < 0.001$), predominantly female (71% vs. 69%), and had sustained less hip fractures (hip fracture/vertebral fracture/non-hip non-vertebral fracture 9%/ 6%/ 85% vs. 22%/ 8%/ 70%; $p < 0.001$). There was no significant difference in the number of female patients who were referred to the FLS or not, $p = 0.229$.

Of these 856 patients referred, 709 agreed to be further investigated for the presence of secondary factors for bone fragility and were included in the study. These were 196 men and 513 women, with a mean age of 67.1 ± 10.2 years (range 50.0 – 94.0 years). Sixty-one (9%) had a hip fracture, 40 (6%) a clinical vertebral fracture, and 608 (86%) a non-hip/non-vertebral (NH/NV) fracture.

**Figure 1**

Flowchart of inclusion of patients in the study after presenting to the Emergency Room of the Leiden University Medical Center with a recent fracture.

ER Emergency Room; FLS Fracture Liaison Service; GP General Practitioner

Two-hundred and one patients (28%) had osteoporosis, 391 (55%) had osteopenia and 117 (17%) had normal Bone Mineral Density (BMD). Data on FRAX clinical risk factors for fracture and/or laboratory data were incomplete in 23 patients, so that 686 patients were included in the final analysis, 385 of whom had osteopenia and 102 normal BMD.

After stratification of patients according to BMD, there were significant differences in mean age (64.7 ± 9.9 years vs. 66.0 ± 9.8 years vs. 70.5 ± 10.2 years, $p < 0.001$), gender distribution (34% vs. 29% vs. 17% male patients, $p = 0.001$) and BMI (28.2 ± 5.1 vs. 26.7 ± 4.1 vs. 24.3 ± 3.8 , $p < 0.001$) between groups of patients with respectively normal BMD, osteopenia and osteoporosis. There was no difference in biochemical parameters, number of patients with a previous fracture or with a history of parental hip fracture between the three BMD groups; Table 1.

Table 1. Characteristics of patients with normal BMD, osteopenia and osteoporosis

	Normal BMD (n = 102)	Osteopenia (n = 385)	Osteoporosis (n = 199)	p-value
Age (years)	64.7 ± 9.9	66.0 ± 9.8	70.5 ± 10.2	< 0.001
Male/female (%)	35/67 (34%/66%)	113/272 (29%/71%)	34/165 (17%/83%)	0.001
BMI (kg/m ²)	28.2 ± 5.1	26.7 ± 4.1	24.3 ± 3.8	< 0.001
Previous Fracture (%)	41 (40%)	163 (42%)	83 (42%)	0.918
Parental Hip Fracture (%)	10 (10%)	50 (13%)	28 (14%)	0.560
FRAX score major fracture	5.2 ± 0.6	8.9 ± 0.3	17.0 ± 0.7	< 0.001
FRAX score hip fracture	0.5 ± 0.3	2.2 ± 0.2	7.3 ± 0.6	< 0.001
Laboratory data:				
Calcium (mmol/L)	2.40 ± 0.12	2.41 ± 0.12	2.41 ± 0.11	0.648
Creatinine (μmol/L)	74.4 ± 15.3	74.7 ± 19.1	72.3 ± 26.4	0.422
PTH (pmol/L)	4.0 ± 2.4	4.4 ± 2.8	4.9 ± 4.2	0.111
25-OH D (nmol/L)	62.7 ± 36.6	57.5 ± 28.2	58.4 ± 30.5	0.677
DXA measurements:				
LS BMD (g/cm ²)	1.11 ± 0.12	0.95 ± 0.12	0.80 ± 0.14	< 0.001
T-score LS	0.5 ± 1.0	-1.0 ± 1.0	-2.3 ± 1.2	< 0.001
FN BMD (g/cm ²)	0.86 ± 0.10	0.70 ± 0.07	0.58 ± 0.08	< 0.001
T-score FN	-0.1 ± 0.7	-1.5 ± 0.5	-2.5 ± 0.6	< 0.001

BMI Body Mass Index; DXA Dual Energy X-Ray Absorptiometry; LS Lumbar Spine; FN Femoral Neck; BMD Bone Mineral Density

The majority of patients had a non-hip/non-vertebral (NH/NV) fracture [n = 586 (85%)], 60 patients (9%) had a hip fracture, and 40 patients (6%) had a clinical vertebral fracture. The most prevalent NH/NV fracture was a wrist fracture [n = 221 (32%)] followed by a fracture of the proximal humerus [n = 91 (13%)] and of the ankle [n = 79 (12%)]. Forty patients (67%) with a hip fracture had a secondary factor for bone fragility, 28 patients (70%) with a vertebral fracture and 322 patients (55%) with a NH/NV fracture; $p = 0.049$.

A similar distribution of fractures was observed in patients with normal BMD: 6 (6%) hip fracture, 4 (4%) clinical vertebral fracture and 92 (90%) NH/NV fracture, osteopenia: 33 (9%) hip fracture, 19 (5%) vertebral fracture and 333 (86%) NH/NV fracture, and in patients with osteoporosis: 21 (11%) hip fracture, 17 (9%) vertebral fracture and 161 (81%) NH/NV fracture.

Secondary factors identified by clinical risk factors using FRAX

In patients with normal BMD, 13 patients (13%) used ≥ 3 units of alcohol a day, 11 (11%) were currently using or had previously used glucocorticoids, 15 (16%) were active smokers and 3 (3%) had rheumatoid arthritis; Table 2. Eight patients (9%) had a history

Table 2. Prevalence of secondary factors for bone fragility in patients with a recent fracture grouped according to gender and BMD

	Male (n = 182)	Female (n = 504)	Normal BMD (n = 102)	Osteopenia (n = 385)	Osteoporosis (n = 199)	Total number patients (n = 686)
FRAX clinical risk factors:						
Smoking (%)	42 (23%)	80 (16%)	15 (16%)	64 (17%)	43 (22%)	122 (18%)
Use of > 3 IU alcohol (%)	30 (17%)	49 (10%)	13 (13%)	44 (11%)	22 (11%)	79 (12%)
Glucocorticoids (%)	14 (8%)	66 (13%)	11 (11%)	42 (11%)	27 (14%)	80 (12%)
Rheumatoid arthritis (%)	2 (1%)	20 (4%)	3 (3%)	11 (3%)	8 (4%)	22 (3%)
Early menopause (%)	-	96 (19%)	13 (13%)	45 (12%)	38 (19%)	96 (14%)
Laboratory based factors:						
Chronic kidney disease (%)	15 (8%)	77 (15%)	10 (10%)	53 (14%)	29 (15%)	92 (13%)
MGUS (%)	25 (14%)	65 (13%)	5 (5%)	46 (12%)	39 (20%)	90 (13%)
1° hyperparathyroidism (%)	-	7 (1%)	1 (1%)	4 (1%)	2 (1%)	7 (1%)
2° hyperparathyroidism (%)	7 (4%)	35 (7%)	3 (3%)	25 (7%)	14 (7%)	42 (6%)
Hyperthyroidism (%)	1 (1%)	12 (2%)	1 (1%)	7 (2%)	5 (3%)	13 (2%)
Hypogonadism (%)	8 (4%)	-	1 (1%)	6 (2%)	1 (1%)	8 (1%)
Patients with > 1 factor (%)	93 (51%)	297 (59%)	48 (47%)	219 (57%)	123 (62%)	390 (57%)

IU International Unit; MGUS Monoclonal Gammopathy of Undetermined Significance

of parental hip fracture and 41 (40%) had sustained one or more previous fractures. The median FRAX 10-year fracture probability was 5.2% for a major osteoporotic fracture and 0.5% for a hip fracture, and was 7.3% and 2.2% respectively without inclusion of femoral neck BMD in the calculation.

In patients with osteopenia, 44 patients (11%) used more than 3 units of alcohol per day, 42 (11%) were currently using or had previously used glucocorticoids, 64 (17%) were active smokers, and 11 (3%) had rheumatoid arthritis; Table 2. 50 patients (13%) had at least one parent with a history of a hip fracture and 163 patients (42%) had sustained a previous fracture.

The median 10-year fracture probability as calculated by FRAX was 8.9% for a major osteoporotic fracture and 2.2% for a hip fracture. Without inclusion of femoral neck BMD in the calculation, the median 10-year fracture probability was 9.4% for a major osteoporotic fracture and 2.8% for a hip fracture.

In patients with osteoporosis, 22 patients (11%) used ≥ 3 units of alcohol a day, 27 (14%) were currently using or had previously used glucocorticoids, 43 (22%) were active smok-

ers and 8 (4%) had rheumatoid arthritis; Table 2. Twenty-eight patients (14%) had a history of parental hip fracture, and 83 (42%) had sustained one or more previous fractures.

The median 10-year fracture probability with inclusion of femoral neck BMD was 17% for a major osteoporotic fracture and 7.3% for a hip fracture, and respectively 16% and 6.2% without inclusion of femoral neck BMD in the calculation.

There was a significant difference in the 10-year FRAX probability for fracture between patients with normal BMD, osteopenia or osteoporosis, with or without inclusion of femoral neck BMD measurements; Table 1. A similar number of patients with normal BMD, osteopenia or osteoporosis had an underlying factor for bone fragility as identified by clinical risk factors for fracture using FRAX (41 patients with normal BMD (40%) vs. 164 patients with osteopenia (43%) vs. 95 patients with osteoporosis (48%); $p=0.381$). There was no difference in the prevalence of any individual clinical risk factor studied between groups; Fig. 2A.

There was a significant difference in the 10-year FRAX probability for fracture calculated with BMD values between patients with ≥ 1 secondary factor and patients without (12.0% vs. 8.5%; $p < 0.001$), and in the 10-year FRAX probability without BMD values (13.0% vs. 8.3% ; $p < 0.001$). Interestingly, there was a significant relationship between the cumulative number of factors for bone fragility and the 10-year FRAX probability

A

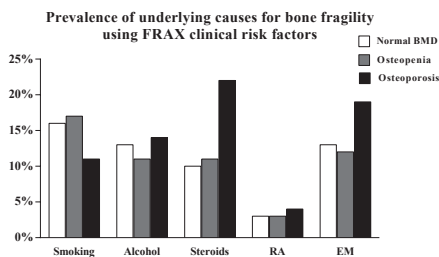


Figure 2A

No significant difference in the prevalence of underlying secondary factors for bone fragility between patients with normal BMD (white bars) osteopenia (grey bars) or osteoporosis (black bars) using FRAX. RA rheumatoid arthritis; EM early menopause.

B

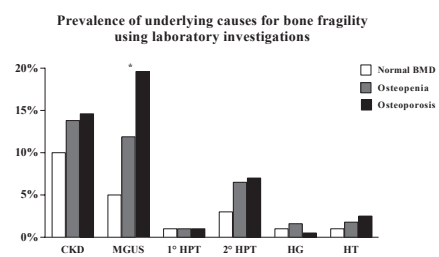


Figure 2B

Prevalence of underlying secondary factors for bone fragility by laboratory investigations in patients with normal BMD (white bars), osteopenia (grey bars) and osteoporosis (black bars). Significantly different prevalence of MGUS in patients between the groups. CKD chronic kidney disease; MGUS Monoclonal Gammopathy of Undetermined Significance; 1° HPT primary hyperparathyroidism; 2° HPT secondary hyperparathyroidism; HG hypogonadism; HT hyperthyroidism.

* $p = 0.001$

($r = 0.336$, $p < 0.001$), and in the 10-year FRAX probability calculated without BMD values ($r = 0.359$, $p < 0.001$). After stratification for BMD status, this relationship remained for normal BMD ($r = 0.286$, $p = 0.004$ for FRAX with BMD and $r = 0.456$, $p < 0.001$ for FRAX without BMD), osteopenia ($r = 0.313$, $p < 0.001$ for FRAX with BMD and $r = 0.311$, $p < 0.001$ for FRAX without BMD) and osteoporosis ($r = 0.361$, $p < 0.001$ for FRAX with BMD and $r = 0.346$, $p < 0.001$ for FRAX without BMD).

Secondary factors identified by laboratory investigations

Laboratory investigations identified an underlying factor for bone fragility in 18 (18%) of patients with normal BMD, in 112 (29%) of patients with osteopenia and in 69 (35%) of patients with osteoporosis.

In patients with normal BMD, chronic kidney disease was diagnosed in 10 patients (10%), all of whom had CKD stage III. One patient (1%) had primary hyperparathyroidism and 3 (3%) had secondary hyperparathyroidism (associated with low 25-OH vitamin D levels in 2, and combined vitamin D deficiency and renal failure in 1). One patient (1%) had hyperthyroidism, 1 male patient had hypogonadism and 5 (5%) patients were diagnosed with MGUS.

In patients with osteopenia, chronic kidney disease was diagnosed in 53 patients (14%), of whom 49 had stage III CKD and 4 had stage IV CKD. Four patients (1%) had primary hyperparathyroidism and 25 (7%) had secondary hyperparathyroidism (associated with low 25-OH vitamin D levels in 15, and combined vitamin D deficiency and renal failure in 8). Seven patients (2%) had hyperthyroidism (5 subclinical), 6 men had hypogonadism and 46 (12%) patients were diagnosed with MGUS.

In patients with osteoporosis, chronic kidney disease was diagnosed in 29 patients (15%), of whom 26 had stage III CKD and 3 had stage IV CKD. Two patients (1%) had primary hyperparathyroidism and 14 had secondary hyperparathyroidism (associated with low 25-OH vitamin D levels in 7, and combined vitamin D deficiency and renal failure in 7). Five patients (3%) had hyperthyroidism (2 subclinical), 1 male patient had hypogonadism and 39 (20%) patients were diagnosed with MGUS.

All underlying factors for bone fragility identified by laboratory investigations were equally prevalent in patients with normal BMD, osteopenia or osteoporosis, except for MGUS (5% vs. 12% vs. 20%; $p = 0.001$); Fig. 2B. One patient with osteoporosis was diagnosed as having multiple myeloma.

The odds of a patient with osteopenia having an MGUS were 2.71 times higher than those of a patient with normal BMD, and the odds of a patient with osteoporosis having an MGUS were 4.81 times higher than a patient with normal BMD. There was no association between BMD and the odds for any other factor for bone fragility; Table 3.

Table 3. Odds-ratio's for factors for increased bone fragility in patients with osteoporosis and osteopenia, compared with patients with normal BMD

	Odds-ratio [95% CI]	p-value
FRAX:		
Smoking	1.610 [0.795-3.259]	0.186
	1.086 [0.578-2.038]	0.798
Use of > 3 IU alcohol	0.916 [0.413-2.033]	0.829
	0.858 [0.436-1.689]	0.657
Glucocorticoids	1.454 [0.645-3.273]	0.367
	1.089 [0.530-2.238]	0.817
Rheumatoid arthritis	1.135 [0.263-4.899]	0.865
	0.952 [0.252-3.605]	0.942
Early menopause	1.660 [0.762-3.613]	0.202
	0.958 [0.470-1.952]	0.905
Laboratory investigations:		
Chronic kidney disease	1.196 [0.510-2.805]	0.681
	1.532 [0.713-3.290]	0.274
MGUS	4.805 [1.745-13.231]	0.002
	2.713 [1.037-7.098]	0.042
1° hyperparathyroidism	2.580 [0.156-42.571]	0.508
	1.808 [0.170-19.278]	0.624
2° hyperparathyroidism	2.212 [0.574-8.518]	0.249
	2.470 [0.708-8.613]	0.156
Hypogonadism	1.330 [0.068-25.847]	0.851
	2.135 [0.239-19.096]	0.498
Hyperthyroidism	1.883 [0.194-18.316]	0.586
	1.822 [0.213-15.609]	0.584

For every risk factor listed, odds-ratio's refer to patients with osteoporosis at the top line and to patients with osteopenia at the second line. Patients with normal BMD are used as reference.

IU Internal Unit; MGUS Monoclonal Gammopathy of Undetermined Significance

Relationship between number of secondary factors for bone fragility and BMD measurements

390 patients (57%) had one or more underlying secondary factor for bone fragility. The majority of patients ($n = 205$ [30%]) had one underlying factor, 122 patients (18%) had two and 63 (9%) had three or more factors. 38% of all observed underlying factors were identified by laboratory investigations, and 41% of all factors were reversible. There was an inverse relationship between the number of underlying factors for bone fragility per patient and mean femoral neck BMD values ($r = -0.215$, $p < 0.001$), which persisted after adjusting for age, gender and BMI ($r = -0.192$, $p < 0.001$).

In patients with normal BMD, 29 patients (28%) had one underlying factor, 13 patients (13%) had two factors and 6 (6%) had three or more factors. In patients with osteopenia,

the majority of patients ($n = 122$ [32%]) had one underlying factor, 69 patients (218%) had two and 28 (17%) had three or more factors. In patients with osteoporosis, 54 patients (327%) had one secondary factor for bone fragility, 40 patients (20%) had two factors and 29 (15%) had three or more factors.

Vitamin D deficiency

Vitamin D deficiency was prevalent in 292 (43%) of the 686 patients, of whom 97 had serum vitamin D levels < 25 nmol/L. Vitamin D deficiency was documented in 43 patients (42%) with normal BMD, in 166 patients (43%) with osteopenia and in 83 patients (42%) patients with osteoporosis.

DISCUSSION

Our data from this study demonstrate that 57% of patients who recently sustained a fracture and who were subsequently referred to our Fracture Liaison Service, had one or more underlying secondary factor for bone fragility. Interestingly, these secondary factors were equally prevalent in our cohort of patients with a recent fracture whether they had normal BMD, osteopenia or osteoporosis (47% vs. 57% vs. 62% respectively; $p = 0.05$.) Our data also show a significant inverse relationship between the number of underlying factors for bone fragility and femoral neck Bone Mineral Density (BMD). The most prevalent underlying factors for bone fragility were smoking (18%), chronic kidney disease (13%) and MGUS (13%). Of clinical relevance is that 41% of all documented secondary factors for bone fragility were reversible (hyperthyroidism, primary and secondary hyperparathyroidism, hypogonadism in men, or potentially reversible such as excessive alcohol use and smoking). This may hold significant clinical implications in the management of these patients as reversal of these factors may be associated with an improvement in bone strength, thus contributing in time to a decrease in bone fragility. In addition, 43% had serum levels of 25-OH vitamin D < 50 nmol/L.

The prevalence of secondary factors for bone fragility has been previously reported in patients with a recent fracture [13, 15]. Although the majority of previously published data studied the prevalence of underlying factors for bone fragility in patients with osteoporosis [13–15, 23–27], our findings suggest a similar prevalence of these factors regardless of BMD measurements after a recent fracture. A novel approach we pursued in this analysis was to compare the prevalence of underlying factors for bone fragility between groups of patients with normal BMD, osteopenia or with osteoporosis in our cohort of 686 patients aged ≥ 50 years who had sustained a recent fracture.

As expected, the FRAX 10-year probability for a fracture was significantly different in patients with normal BMD, osteopenia or osteoporosis. This was also the case when femoral neck BMD was not used in the calculation of the FRAX. Our data also demonstrate that patients with one or more secondary factor for bone fragility had a higher 10-year FRAX fracture probability compared to patients with no documented secondary factor(s). Interestingly there was a positive relationship between cumulative number of secondary factors for bone fragility and FRAX independently of BMD status, which suggests that FRAX may not fully capture the contribution of the cumulative effect of these factors on fracture risk.

Our study has strengths as well as limitations. One of its main strengths is that all fracture patients were identified at source at the outpatient clinics of the Departments of Traumatology or Orthopaedic Surgery by a dedicated fracture nurse from our FLS resulting in the successful referral of > 50% patients to our FLS for screening for osteoporosis. Over the last decade, there has been an increasing drive to develop and implement FLSs on an international scale for the secondary prevention of fractures [28]. These FLSs ensure that future fracture risk is assessed in all patients who have sustained a recent fracture, including the risk of falling [29]. These FLSs also secure that treatment with bone modifying agents is initiated if and when required. Previous studies demonstrated that the implementation of an FLS was cost-effective and effectively reduced mortality and the incidence of subsequent non-vertebral fractures [8, 30].

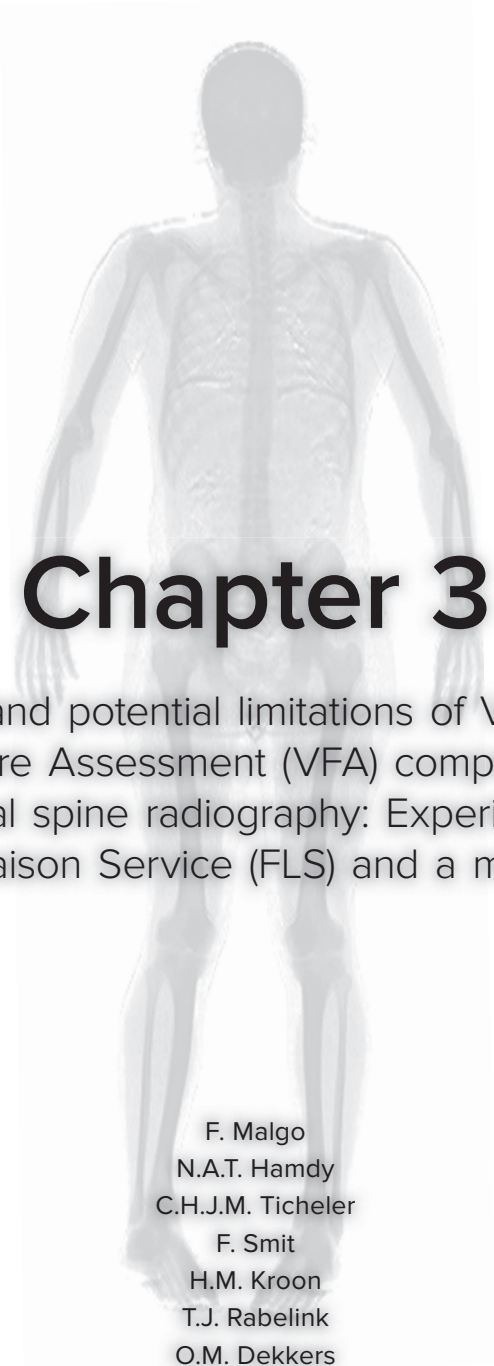
A further strength of the study is the availability of data on clinical risk factors using FRAX, of BMD data and of laboratory data on the most common secondary factors for bone fragility. A main limitation of the study is that due to regional hospital policy, the majority of patients who presented to our Emergency Room with a hip fracture were transferred to other hospitals in the region for primary fracture care, which could have influenced patient characteristics, especially age. The limited screening for monoclonal gammopathy of undetermined significance (MGUS) may also be considered as a limitation of our study, as data may have been confounded by the bias of the treating physician's decision to screen patients at higher risk for underlying secondary factors for bone fragility. However, this would have led to an underestimate rather than an overestimate of the prevalence of an MGUS. LS BMD measurements were not adjusted for the presence of degenerative changes, which might explain the absence of a relationship between the number of underlying factors for bone fragility and LS BMD. However, we also found no correlation between the number of underlying factors and LS BMD in patients stratified by age groups (data not shown).

Our data, demonstrating a high prevalence of secondary factors for bone fragility, independently of BMD status, in a cohort of patients who had sustained a recent fracture, hold significant clinical implications in the management of these patients, as nearly half of these factors were potentially reversible. Our findings suggest that screening for underlying secondary factors for bone fragility should be considered in the setting of Fracture Liaison Services, not only in patients with osteoporosis but also in those with osteopenia or normal BMD. Whether reversing secondary factors for bone fragility would result in more optimal secondary prevention of fractures remains to be established by long-term studies.

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Chapter 3

Value and potential limitations of Vertebral Fracture Assessment (VFA) compared to conventional spine radiography: Experience from a Fracture Liaison Service (FLS) and a meta-analysis

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ABSTRACT

Summary:

We evaluated the value of VFA in the identification of vertebral fractures using a retrospective study and a meta-analysis. Performance of VFA was adequate in the meta-analysis although this was not demonstrated in our center. We recommend to check the performance of VFA tools before exclusively relying on this tool.

Introduction:

Vertebral fractures are traditionally diagnosed using conventional radiographs of the spine. Vertebral Fracture Assessment (VFA) has been advocated as an alternative tool in the diagnosis of these fractures.

Methods:

We conducted a retrospective study as well as a systematic review and a meta-analysis to evaluate the performance of VFA compared to conventional spinal radiography in patients who had sustained a fracture and thus at risk for osteoporosis. A risk of bias analysis was also performed.

Results:

The diagnostic study included 542 patients (25% male) with fractures. The sensitivity of low-radiation VFA to detect a patient with a vertebral fracture $\geq$ Genant grade 2 was 0.77 and its specificity 0.80. 297(55%) patients had ≥ 1 and 135(25%) ≥ 3 unevaluable vertebrae. The systematic review identified 16 studies including a total of 3238 subjects (19% male) with a mean age range of 45 to 74 years. Seven studies had a low risk of bias and 9 had an intermediate risk, mainly due to not consecutively including patients. The pooled sensitivity of VFA to detect a patient with a vertebral fracture $\geq$ Genant grade 2 was 0.84 (95% CI, 0.72–0.92) and specificity 0.90 (95% CI, 0.84–0.94).

Conclusions:

Our findings from the meta-analysis suggest an adequate performance of VFA for the detection of vertebral fractures. However, we could not demonstrate these findings in our center, especially the specificity. Our data advocate caution with exclusively relying on VFA in the assessment of vertebral fractures without identifying performance and potential limitations of the technique.

INTRODUCTION

Vertebral fractures are associated with increased mortality and morbidity, and decreased quality of life, and the incidence of these fractures increases with age (1–4). The prevalence and grade of severity of vertebral fractures have also been shown to be predictive for the risk of new vertebral and non-vertebral fractures, independently of Bone Mineral Density (BMD) measurements (5–7). However, vertebral fractures remain often underdiagnosed despite their clear value in the assessment of fracture risk (8, 9). Conventional spine radiography is traditionally used in the evaluation of vertebral fractures and is considered to be the “gold standard” for detection of these fractures and their grading using the semi-quantitative method of Genant (10). Vertebral Fracture Assessment (VFA) is performed using images obtained by bone densitometers in the same session as Bone Mineral Density measurements are performed to screen for osteoporosis. The VFA technique enables the acquisition of a patient-friendly alternative to conventional radiographs for the assessment of vertebral fractures in a one stop diagnostic test (11). Other advantages of VFA include lower radiation exposure and possibly lower costs. On the basis of available data, VFA has indeed already been incorporated in a number of clinical guidelines replacing conventional radiography for the assessment of prevalent vertebral fractures and thus for the risk of fracture (12, 13).

However, the advantage of lower radiation doses used in certain bone densitometry scanners are associated with the drawback of poor image quality, and thus of potential poor visualization of the contours of the vertebrae and this, which could lead to misclassification of fractures or the ascertaining of a vertebra as non-evaluable leading to an inaccurate estimation of fracture risk. A standard protocol or technique for performing VFA has never actually been developed and the majority of published studies compare the performance of VFA to that of conventional spine radiographs mostly in diverse patient populations, often consisting of small numbers and mostly using different hardware and radiation protocols.

The aims of our study were twofold: first to evaluate the performance of VFA compared to conventional spine radiography in our Fracture Liaison Service (FLS) to assess whether we could replace conventional radiographs by VFA in the diagnosis of vertebral fractures in patients evaluated for osteoporosis after a recent fracture. Second, to systematically review all published literature on the performance of VFA compared to conventional spine radiography in patients evaluated for suspected osteoporosis and to perform a meta-analysis on these data.

METHODS

Vertebral Fracture Assessment: VFA compared to conventional radiography

Study design

This was a retrospective study evaluating the performance of low-radiation single energy x-ray absorptiometry VFA for the detection of vertebral fractures compared to conventional radiography of the spine, in a cohort of consecutive men and women aged 50 years or older who had sustained a fracture between June 2012 and June 2014, and who were assessed for osteoporosis according to screening protocols used in the FLS of the Leiden University Medical Center (14). In these protocols, all patients attending the FLS are screened, diagnosed and treated for osteoporosis where required, and data collected at source in a database. Because of the nature of the study, the Medical Ethics Committee of the Leiden University Medical Center deemed that no written informed consent was required.

For the purpose of this analysis, only patients with available data on both VFA and conventional radiography were included in the study. The following data were retrieved from the database: age, gender, height, weight, a detailed fracture history, family history of osteoporosis, a list of current medication and history of use of bone modifying agents were obtained.

Bone Mineral Density measurements

Bone Mineral Density was measured at the lumbar spine (L1–L4) and at the left and right femoral neck by dual-energy X-ray absorptiometry (DXA) using Hologic QDR 4500 (Hologic, Bedford, MA, USA). NHANES III reference values compatible with reference values for the Dutch population were used to calculate T scores. The diagnosis of osteoporosis, osteopenia or normal BMD was established using the World Health Organization criteria.

Vertebral Fracture Assessment (VFA)

In addition to the BMD measurements, single energy x-ray lateral VFA images of the spine (T4–L4) were obtained by a dedicated technician with the patient lying in supine position and a cushion supporting the knees. The effective radiation dose of a VFA scan received by the patient is typically 3 microSievert.

Conventional radiography of the spine

Antero-posterior (thoracic spine), postero-anterior (lumbar spine) and lateral conventional radiographs of the thoracic and lumbar spine were performed by a radiology

technician using a standardized protocol, with the detector centralized on Th7 for the thoracic spine and on L3 for the lumbar spine.

Assessment of vertebral fractures using VFA and conventional radiography of the spine

The presence of vertebral fractures was assessed on VFA images using Hologic QDR Physician Viewer software. The software generates six points on each vertebral end-plate which were then manually adjusted by a dedicated technician as required. In the majority of vertebrae (more than 90%), manual adjustment of the automatically placed points had to be performed by a trained laboratory technician. Anterior, middle and posterior corporal heights were calculated automatically. Following this quantitative evaluation, the software further used the criteria for the classification of vertebral fractures as described by Genant (10). Analyses were performed on a per-person basis. A vertebral fracture was defined as Genant grade 2 or more. All routinely generated reports of conventional radiographs performed as part of the protocol used in the FLS were retrieved from the patient's electronic medical records. In addition, one of the authors (F.M.) further assessed all radiographs for the presence and grading of vertebral fractures. Both observers were blinded to the VFA findings. Vertebral fractures were classified according to Genant grading system: grade 1 for an anterior, mid or posterior reduction of 20%–25% in vertebral height; grade 2 for a reduction of 25%–40%; and grade 3 for a reduction of more than 40% in vertebral height. In case of disagreement between radiology reports and evaluation from (F.M.), spine radiographs were evaluated by an experienced musculoskeletal radiologist (H.K.), whose evaluation was decisive and used in the analysis. In addition, a randomly selected sample of 20% of the remaining patients was also evaluated by (H.K.) in order to validate the classification of vertebral fractures based on the combined report of the FLS charts and (F.M.), which yielded a kappa of 0.82.

Systematic review of literature

Search strategy

We designed a search strategy in collaboration with a trained librarian for studies that primarily focussed on the diagnostic accuracy of Vertebral Fracture Assessment (VFA) compared to conventional radiographs of the spine in the diagnosis and grading of vertebral fractures in patients at risk for osteoporosis. The search was conducted in Pubmed, MEDLINE, EMBASE and Web of Science and included all published articles on the topic up to June 10th, 2016. All relevant keywords were used, including free text words. The complete search strategy is provided as Supplemental Data.

Eligibility criteria and data extraction

Only original articles written in English were included. Inclusion criteria were: 1) comparison between VFA and spine radiographs performed for the diagnosis of vertebral fractures with reported data on sensitivity and specificity, 2) suspicion of osteoporosis as indication for the assessment of vertebral fractures, 3) use of the Genant's or ABQ's method to assess the presence of vertebral fractures in radiography and 4) patients aged ≥ 18 years. Studies that reported patients with diseases of the spine such as ankylosing spondylitis or patients recruited from the general population were not eligible.

Articles were assessed by two independent investigators (F.M. and N.M.A-D), first by screening for eligibility for inclusion in the analysis by title and abstract. Selected articles were further assessed in detail. Disagreements were resolved by consensus.

The following data were extracted from all selected publications: number of patients studied, age and gender distribution, hardware used for VFA and DXA, study inclusion criteria, method of assessment of vertebral fractures, prevalence of vertebral fractures, and sensitivity and specificity of VFA.

Risk of bias assessment

The following characteristics of the study design were evaluated for each published study used in the review to assess the risk of bias:

- 1) Inclusion of patients; were consecutive patients who had conventional spine radiographs included in the study or were only selected patients included? Inclusion of consecutive patients was considered a low risk of bias.
- 2) Definition of vertebral fractures used in the study. Analysis of data using a definition of Genant 2 or higher for vertebral fractures was considered a low risk of bias (10).
- 3) Clear and adequate description of method used to assess vertebral fractures in VFA and conventional radiography of the spine. Complete description of methodology for the assessment of vertebral fractures was considered a low risk of bias.
- 4) Blinding of the examiner who examined VFA for the outcome of the spine radiographs. Blinded assessment was considered a low risk of bias.

For each of the 4 elements named above, studies were qualified as adequate, inadequate or not reported.

Statistical analysis

The performance of VFA was calculated using conventional radiography as reference, sensitivity was estimated by the number of true-positive vertebral fractures divided by the number of vertebral fractures identified by conventional radiographs, and specificity was calculated by the number of true-negative vertebral fractures divided by the number of intact vertebrae observed on conventional radiographs. The main outcome

of the meta-analysis was the pooled sensitivity and specificity of VFA. Conventional radiographs of the spine were used as the “gold standard”. The meta-analysis was based on a random effects model and a bivariate approach, and sensitivity and specificity were estimated both per vertebra and per person. Heterogeneity was assumed and explored as recommended by Leeflang et al (15).

RESULTS

Vertebral Fracture Assessment: VFA compared to conventional radiography

Five hundred and forty-two patients [137 (25%) men and 405 (75%) women] were included in the study. Mean age of the population was 67.5 ± 10.1 years (range 50.0 – 92.8), mean BMI was 26.1 ± 4.3 kg/m² and median time between fracture and FLS visit was 2.3 months. Fifty patients (9%) had sustained a fracture of the hip, 25 (5%) of the vertebrae, 188 (35%) of the distal radius, 58 (11%) of the proximal humerus and 61 (11%) of the ankle. The majority of patients had osteopenia ($n = 319$, 59%), 163 (30%) had osteoporosis, and 60 (11%) had a normal Bone Mineral Density; Table 1. On low-radiation VFA, 184 (34%) patients had at least one grade 2 or higher vertebral fracture, of whom 47 had a Genant grade 3 vertebral fracture. These were 56 men and 128 women with

Table 1. Patient characteristics

Age (years)	67.5 $\pm$ 10.1
Male/female	137/405
BMI (kg/m ²)	26.1 $\pm$ 4.3
Time between fracture and VFA (months)	2.3 $\pm$ 0.08
Type of fracture:*	
Vertebra	25 (5%)
Hip	50 (9%)
Proximal humerus	58 (11%)
Ankle	61 (11%)
Distal radius	188 (35%)
DXA-BMD:	
Normal	60 (11%)
Osteopenia	319 (59%)
Osteoporosis	163 (30%)

Values are expressed as mean $\pm$ SD, except for time between fracture and VFA

BMI body mass index; kg/m² kilogram per square meter; VFA vertebral fracture assessment; DXA-BMD bone mineral density measurement

* Fracture for which the patient was referred to FLS

a mean age of 71.4 ± 10.3 years. One hundred and six (58%) patients had osteopenia, 64 (35%) osteoporosis and 14 (8%) had a normal BMD.

Conventional radiographs of the spine identified 132 (24%) patients with $\geq$ one grade 2 or higher vertebral fracture, of whom 47 had a Genant grade 3 vertebral fracture. VFA correctly identified 102 of the 132 patients with a $\geq$ grade 2 vertebral fracture, corresponding to a sensitivity of 0.77 (95% CI, 0.70–0.84). Of the 30 patients who were missed on VFA, 17 had ≥ 1 vertebrae that could not be evaluated by VFA. Of these patients, 3 had a radiological fracture on their radiographs at a vertebral level that could not be evaluated by VFA, and were thus missed. Of the 410 patients without a vertebral fracture on spinal radiographs, 328 were also found not to have a vertebral fracture on VFA corresponding to a specificity of 0.80 (0.76–0.84); Table 2. Interestingly 297 (55%) patients had ≥ 1 vertebrae that could not be evaluated by VFA and 135 (25%) patients had 3 or more unevaluable vertebrae; Figure 1.

The vertebrae that could not be evaluated by VFA or the patients that were misclassified by VFA were independent of the type of the recently sustained fracture or of the time between the recent fracture and the FLS visit.

Table 2. Outcome of VFA compared to conventional spine radiography for the detection of vertebral fractures $\geq$ grade 2

		Radiography of the spine	
		Vertebral fracture	No vertebral fracture
VFA	Vertebral fracture	102	82
	No vertebral fracture	30	328
	Total	132	410

VFA vertebral fracture assessment

Search strategy

The search strategy for the systematic review of the literature yielded 694 articles (201 from PubMed, 167 from MEDLINE, 203 from Embase and 123 from Web of Science). 270 studies were unique and potentially relevant, and were further assessed for eligibility. 241 studies were excluded on the basis of title and abstract, 14 were review papers and 1 was a Position Paper, 2 studies were performed in a paediatric population, 1 study was written in French and 1 study could not be obtained. Twenty-nine studies were acquired for full assessment. Of these, 4 were excluded because there was no comparison between VFA and conventional radiography, 2 studies did not report performance parameters, 3 studies included patients with a rheumatologic disorder, 1 study included patients from the general population and 1 study was an autopsy study; Figure 2.

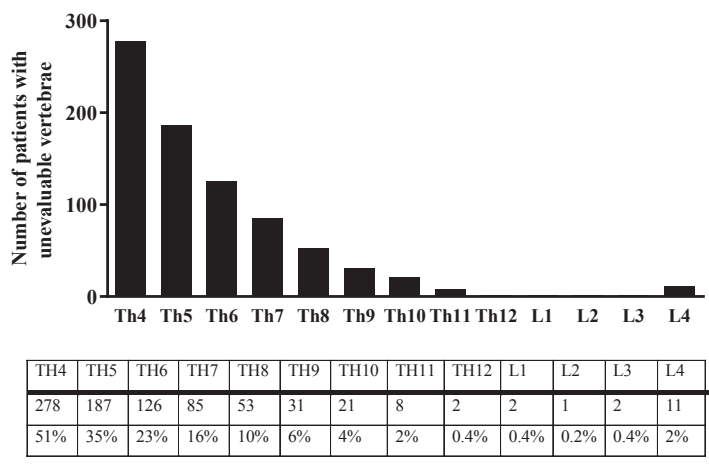


Figure 1
Number (%) of patients with vertebrae that could not be evaluated by VFA.

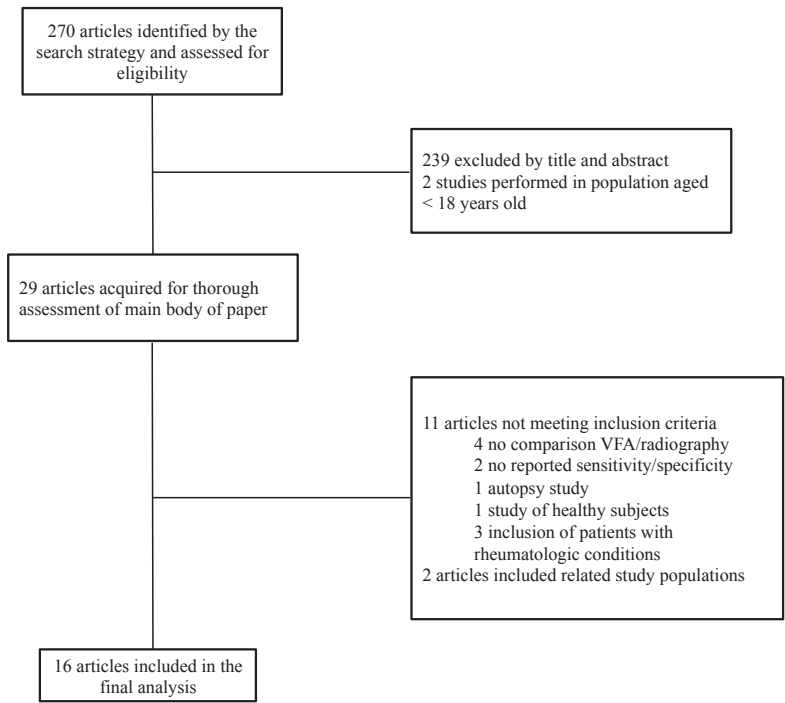


Figure 2
Flowchart of selection of articles for systematic review and meta-analysis
VFA Vertebral Fracture Assessment

Table 3. Characteristics of studies included in meta-analysis

Author, Publication year	Number of patients (m/f)	Age (range)	Hardware	Inclusion criteria	Analysis method	VF prevalence (%)	Outcome parameters (sensitivity/specificity)
Bazzocchi (2012)	68 (38/30)	58.1 (32-83)	Lunar DXA	Suspicion of osteoporosis, glucocorticoid treatment, organ transplantation)	Genant	Vertebra ≥ grade 1 4.9 ≥ grade 2 1.8	Vertebra ≥ grade 1 70.0 98.3 ≥ grade 2 53.8 99.6
Binkley (2005)	80 (0/80)	72.8 (61-84)	Lunar Prodigy	PM women participated in osteoporosis treatment studies, or having clinical BMD measurement	Genant	Person ≥ grade 1 38.2 Vertebra ≥ grade 1 4.8 ≥ grade 2 2.2	Person ≥ grade 1 73.1 90.5 Vertebra ≥ grade 1 70 96 ≥ grade 2 94 99
Chapurlat (2006)	85 (0/85)	71 (NR)	Hologic Delphi	PM women with screening BMD measurement or VF on radiography	Genant	Person ≥ grade 1 50 Vertebra ≥ grade 1 9.9	Person ≥ grade 1 69 74 Vertebra ≥ grade 1 NR NR
Damiano (2006)	136 (0/136)	69.1 (37-96)	Hologic Delphi W	PM women with indication for spinal radiography in rheumatology department	Genant	Person ≥ grade 1 50 Vertebra ≥ grade 1 9 ≥ grade 2 6	Person ≥ grade 1 69 74 Vertebra ≥ grade 1 82.8 98.3 ≥ grade 2 82.8 99.3
Deleskog (2016)	35 (5/30)	67.5 (NR)	Hologic Discovery A QDR	Patients referred for Forsteo treatment	Genant	Person ≥ grade 1 45.8 Vertebra ≥ grade 1 39 ≥ grade 2 29	Person ≥ grade 1 94.1 83.0 Vertebra ≥ grade 1 76 87 ≥ grade 2 72 92
					Person	Person	Person

Table 3. Characteristics of studies included in meta-analysis (continued)

Author, Publication year	Number of patients (m/f)	Age (range)	Hardware	Inclusion criteria	Analysis method	VF prevalence (%)	Outcome parameters (sensitivity/specificity)
Diacinti (2012)	350 (81/269)	60.6 (28-85)	Lunar iDXA	Patients referred by GP for osteoporosis and from a study of HIV-related osteoporosis	Genant	Vertebra ≥ grade 1 5.1	Vertebra ≥ grade 1 97.0 99.9
Diacinti (2012)	930 (0/930)	62.4 (46-85)	Hologic QDR-4500A	PM women referred by GP for evaluation of osteoporosis	Genant	Person ≥ grade 1 36 Vertebra ≥ grade 1 3.7	Person ≥ grade 1 96.8 98.7 Vertebra ≥ grade 1 97.9 99.8
Ferrar (2000)	Ref: 123 (0/123)	Ref: 66.6 (56-88)	Hologic QDR-4500 A	Ref: PM women from prospective population-based study	Melton	Person ≥ grade 1 27 Ref: Vertebra ≥ grade 1 1.6	Person ≥ grade 1 97.2 98.9 Ref: Vertebra ≥ grade 1 54 99
	OP: 83 (0/83)	OP: 70 (49-87)		OP: Women with VF Excluded scoliosis		Person --- OP: Vertebra ≥ grade 1 32	Person --- OP: Vertebra ≥ grade 1 72 96
Ferrar (2008)	LR: 459 (459)	LR: 68 (55-79)	Hologic QDR-4500 A	PM women recruited from GP Sheffield arm OPUS	ABQ	LR: Vertebra ≥ grade 1 2 ≥ grade 2 NR	LR: Vertebra ≥ grade 1 70.6 85.1 ≥ grade 2 75.7 99.9
						Person ≥ grade 1 11 ≥ grade 2 NR	Person ≥ grade 1 71.2 97.4 ≥ grade 2 74.4 99.5

Table 3. Characteristics of studies included in meta-analysis (continued)

Author, Publication year	Number of patients (m/f)	Age (range)	Hardware	Inclusion criteria	Analysis method	VF prevalence (%)	Outcome parameters (sensitivity/specificity)
Fuerst (2009)	HR: 298 (0/298)	HR: 68 (55-80)	Hologic Delphi (n=99)	PM women attending MBC Sheffield Recent low-trauma fracture, VF, prednisolone >5 mg for 3 months	Genant	HR: Vertebra ≥ grade 1 5 ≥ grade 2 NR	HR: Vertebra ≥ grade 1 79.4 ≥ grade 2 67.1 Person ≥ grade 1 84.3 ≥ grade 2 85.7
Hospers (2009)	250 (60/190)	67.5 (NR)	Lunar Prodigy (n=104)	Multicenter in patients with osteoporosis	Genant	Vertebra ≥ grade 1 4.8 ≥ grade 2 2.5	Vertebra ≥ grade 1 70-73 ≥ grade 2 70-86 Person ≥ grade 1 72-73 ≥ grade 2 77-90
Mazzaferro (2006)	53 (31/22)	62.0 (25-89)	Hologic Discovery	Patients referred to NMP for suspicion primary/secondary OP	Genant	Vertebra ≥ grade 1 5 ≥ grade 2 2.3	Vertebra ≥ grade 1 83.6 ≥ grade 2 94.4 Person ≥ grade 1 NR Vertebra ≥ grade 1 83.7 ≥ grade 2 100
Mazzaferro (2006)	53 (31/22)	45 (23-68)	Hologic QDR-4500A	Renal transplant recipients	Eastell	Person ≥ grade 1 37 Vertebra ≥ grade 1 7.1	Person ≥ grade 1 NR Vertebra ≥ grade 1 83.7 Person ≥ grade 1 70.6

Table 3. Characteristics of studies included in meta-analysis (continued)

Author, Publication year	Number of patients (m/f)	Age (range)	Hardware	Inclusion criteria	Analysis method	VF prevalence (%)	Outcome parameters (sensitivity/specificity)
Rea (2000)	161 (0/161)	64 (49-81)	Hologic QDR-4500A	PM women recruited from GP, and PM women in MBC - T-score ≥ 2 SD - 1 VF Excluded scoliosis	Visual subjective assessment	Vertebra $\geq$ grade 1 11 $\geq$ grade 2 6.9	Vertebra $\geq$ grade 1 77.4 98.4 $\geq$ grade 2 91.9 96.3
Rud (2016)	235 (25/210)	73 (65-90)	Hologic Discovery A	Consecutive patients referred for osteoporosis assessment	Genant	Person $\geq$ grade 1 38 $\geq$ grade 2 26 Vertebra ---	Person $\geq$ grade 1 78.3 89.0 $\geq$ grade 2 97.6 84.9 Vertebra ---
Schousboe (2006)	205 (0/205)	74.2 (65-93)	Hologic Delphi C/W	First 100: all women referred for BMD analysis $\geq$ 65 years After: women $\geq$ 65 years with osteoporosis or osteopenia	Genant	Vertebra $\geq$ grade 1 NR $\geq$ grade 2 (0.8-1.1)	Vertebra $\geq$ grade 1 47-57 99-99 $\geq$ grade 2 52-54 99-99
Vokes (2003)	66 (gender distribution unclear)	64 (19-93)	Lunar Prodigy	Subjects referred for routine BMD measurement	Genant	Person $\geq$ grade 1 NR $\geq$ grade 2 (6.9-7.8) Vertebra ---	Person $\geq$ grade 1 52-63 85-89 $\geq$ grade 2 63-79 93-93 Vertebra ---
						Person $\geq$ grade 2 32	Person $\geq$ grade 2 95 82

Age is expressed as mean (range)

M male; F female; VF vertebral fracture; PM postmenopausal; BMD bone mineral density; NR not reported; GP general practitioner; HIV human immunodeficiency virus; Ref reference population; OP osteoporosis; LR low risk; HR high risk; MBC metabolic bone clinic; NMP nuclear medicine department; SD standard deviation

Eighteen articles met all specified inclusion criteria, two of which reported on related study populations. A total of 16 studies were thus included in the final analysis. Two of these 16 studies included two different populations, namely patients at high and low risk for osteoporosis and/or fractures (16, 17). In keeping with our inclusion criteria, patients recruited from the general population and thus at low risk for osteoporosis were excluded from the analysis ($n = 582$).

Study characteristics

A total number of 3238 subjects were included in the analysis, the vast majority of whom were women ($n = 2626$). The number of subjects per study ranged from 35 (18) to 930 (19) subjects. Mean age of the studied populations ranged from 45 years to 74 years. The youngest included patient was 23 years old (20) and the oldest 96 years old (21). There were seven studies that included both female and male subjects (18, 20, 22–26). One study included both male and female subjects but did not specify the exact gender distribution of subjects who had conventional spinal radiography in addition to VFA (25); Table 3.

All studies included subjects recruited from outpatient clinics, and two studies additionally included patients admitted with a recent vertebral fracture (27) or hip fracture (17). One study solely included patients with radiological evidence of osteoporotic vertebral fractures (16). Two studies used data on VFA and conventional radiography originally collected for another study (23, 28), one used data from osteoporosis treatment studies, and one from an HIV-related osteoporosis study. Three studies reported the inclusion of patients who had recently sustained a fracture (17, 24, 27).

Twelve of the 16 studies used Hologic hardware and five used GE Lunar hardware to acquire VFA scans, with one of the 16 studies acquiring VFA images with either Hologic or GE Lunar technology (29).

Prevalence of vertebral fractures on conventional spine radiography

The prevalence of vertebral fractures $\geq$ grade 1 ranged from 1.8% (22) to 39% (18), the prevalence of patients with a vertebral fracture $\geq$ grade 1 ranged from 6.9% (30) to 100% (16).

Per vertebra analysis

Two studies did not report the VFA sensitivity and specificity per vertebra (25, 26).

The reported sensitivity of VFA to detect a vertebral fracture $\geq$ grade 1 ranged from 46.7% to 98.7%, and from 52.4% to 94.4% to detect a grade 2 or 3 vertebral fracture. The reported specificity of VFA to detect a vertebral fracture $\geq$ grade 1 ranged from 85.1% to 99.9% and the specificity range to detect a vertebral fracture $\geq$ grade 2 was 92% to 99.5%.

Per person analysis

Twelve studies reported VFA parameters per patient basis (17, 19, 20–23, 25–27, 29–31).

The VFA sensitivity range to detect a patient with a $\geq$ grade 1 vertebral fracture was 52% to 97.2% and with a $\geq$ grade 2 vertebral fracture was 62% to 95%. The specificity ranged from 74% to 98.9% to detect a patient with a vertebral fracture $\geq$ grade 1 and ranged from 82% to 99% to detect a patient with a vertebral fracture $\geq$ grade 2.

Risk of bias assessment

Seven studies were classified as having a low risk of bias (17, 19, 22, 23, 26, 29, 30). The other nine studies were classified as having an intermediate risk of bias.

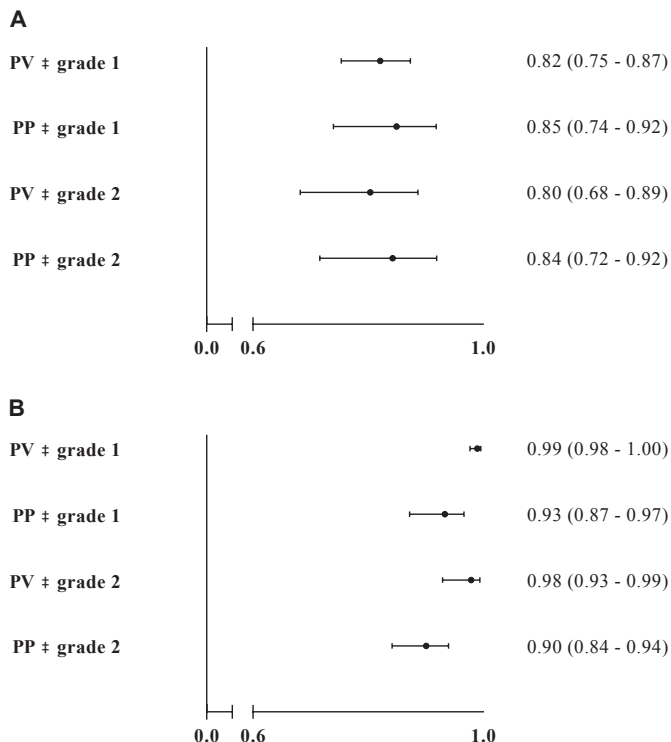


Figure 3

(A) Random effects meta-analysis of sensitivity of VFA to detect vertebral fractures.

PV per vertebra; PP per person

(B) Random effects meta-analysis of specificity of VFA to detect vertebral fractures.

PV per vertebra; PP per person

Twelve studies had no clear consecutive inclusion of patients, 4 studies did not have a per vertebra and per person analysis of only $\geq$ grade 2 vertebral fractures for per vertebra and per person, and 3 and 6 studies did not respectively have a per vertebra and per person analysis. One study did not have a clear description of vertebral fractures and another study lacked clear information about blinding of observers; Supplemental Table 1.

Meta-analysis

In the meta-analysis, sensitivity and specificity were calculated per vertebra and per person; Figure 3. In the per vertebra analysis to detect a vertebral fracture $\geq$ grade 1, sensitivity was 0.82 (95% CI, 0.75–0.87) and specificity was 0.99 (95% CI, 0.98–1.00). In the per person analysis to detect a vertebral fracture $\geq$ grade 1, sensitivity was 0.85 (95% CI, 0.74–0.92) and specificity was 0.93 (95% CI, 0.87–0.97).

The per-vertebra sensitivity of VFA to detect a vertebral fracture $\geq$ grade 2 was 0.80 (95% CI, 0.68–0.89), and specificity was 0.98 (95% CI, 0.93–0.99). The per-person sensitivity of VFA to detect patients with a vertebral fracture $\geq$ grade 2 was 0.84 (95% CI, 0.72–0.92) and specificity was 0.90 (95% CI, 0.84–0.94).

DISCUSSION

We performed a systematic review of the literature and a meta-analysis of published data to evaluate the performance of VFA compared to conventional spine radiography in the identification of vertebral fractures in patients at high risk for osteoporosis. Findings from these data show a sensitivity of 0.82 (95% CI, 0.75–0.87) and specificity of 0.99 (95% CI, 0.98–1.00) on a per vertebra basis, and a sensitivity of 0.85 (95% CI, 0.74–0.92), and specificity of 0.93 (95% CI, 0.87–0.97) on a per person basis. The highly variable sensitivity (47–99%) and specificity (74–100%) between reported studies is likely to be due to the wide age range, variable gender distribution and difference in recruitment of patients (from general practitioners, the outpatient clinics or from an admission ward) between studies. These differences, which were also recognized in a recent systematic review (32), represent a significant limitation in the interpretation and comparison of findings between studies.

Our meta-analysis of available data from published studies show adequate sensitivity and specificity, also when a vertebral fracture was defined as a vertebral fracture $\geq$ grade 2: sensitivity of 0.81 (95% CI, 0.67–0.91) and specificity of 0.98 (95% CI, 0.94–1.00) for per vertebra analysis, and sensitivity of 0.84 (95% CI, 0.72–0.92) and specificity of 0.90 (95% CI, 0.84–0.94) for per person analysis. It would be expected that performance of VFA increased if only vertebral fractures $\geq$ grade 2 were included.

However, intriguingly, the performance of VFA improved when the analysis included vertebral fractures $\geq$ grade 1 rather than only vertebral fractures $\geq$ grade 2. This may be explained by the fact that two of the largest published studies had excellent performance parameters, and provided nearly half of all patients included in the meta-analysis of performance for identifying vertebral fractures $\geq$ grade 1 (19, 23). However, an analysis for the detection of vertebral fractures $\geq$ grade 2 was not performed in these two studies, which may explain the difference in sensitivity and specificity in identifying vertebral fractures $\geq$ grade 1 and $\geq$ grade 2. The risk of bias assessment showed that 7 out of 16 studies had a low risk of bias, and 9 were at moderate risk of bias. It is of note, however, that the majority of these studies did not provide adequate information regarding the inclusion process of patients.

Vertebral Fracture Assessment has become a commonly used tool for the detection of vertebral fractures in the setting of Fracture Liaison Services, clinical care pathways where patients who have recently sustained a fracture are screened for osteoporosis and for potential underlying secondary factors for increased fracture risk. Conventional radiographs of the thoracic and lumbar spine are used as the gold standard for identification of a vertebral fracture. It has been suggested that VFA may represent an attractive alternative to spine radiographs for the detection of vertebral fractures because of simplicity of the technique (using available DXA device) and lower radiation doses than those used in conventional spine radiographs. However, the advantage granted by a lower radiation dose is unfortunately counterbalanced by higher noise rates and therefore lower image quality, often precluding adequate visualization of vertebrae for the presence of a fracture. This may potentially lead to under diagnosis of vertebral fractures or the need for confirmatory spine radiography.

In our FLS, a VFA is performed in all patients at the time of BMD measurements and conventional spine radiography. We performed a retrospective study comparing low-radiation VFA with conventional spine radiography in the detection of patients with vertebral fractures $\geq$ grade 2 in 542 men and women who had recently sustained a fracture. VFA correctly detected 77% of all patients with a vertebral fracture, and correctly identified 80% as having no vertebral fracture. Low-radiation VFA was false positive in 82/410 (20%) patients who had no vertebral fractures on conventional radiography, potentially resulting in over diagnosis and thus initiating unnecessary osteoporosis treatment. Perhaps more worryingly, low-radiation VFA failed to identify a vertebral fracture $\geq$ grade 2 or more in 30 of 132 patients (23%) and more than half of patients had $\geq$ 1 vertebrae that could not be evaluated by VFA, the majority of which were at the upper thoracic spine region (level Th4 and Th5), potentially resulting in under diagnosis and under treatment. Of these, only 3 were missed because the fractured vertebrae were deemed unevaluable by VFA, suggesting poor diagnostic

performance, the precise cause of which is as yet to be identified, rather than just poor visualization due to poor image quality.

Our study has strengths as well as limitations. Its main strength is the large group of consecutive patients of both genders all aged ≥ 50 years who had recently sustained a fracture, and who were uniformly evaluated using our FLS standard protocols. A possible limitation of the study is that the inclusion of 144 patients was precluded by the lack of data on VFA or radiography. Whereas a further limitation could be the theoretical influence of a learning curve to obtain VFA images as this tool was only implemented in our FLS care pathway from 2012 onwards, we found no difference in VFA performance in the first 100 patients compared to the last 100 patients (data not shown). A matter of concern in our study is that the number of patients with ≥ 1 unevaluable vertebrae is rather high, particularly in the upper thoracic region. This problem has been reported in other VFA studies, which led Deleskog and colleagues to suggest that the method was inferior to conventional spinal radiography (18). Notwithstanding, it appears that it may be possible to technically enhance the performance of VFA by methods aiming at improving image quality (thus reducing the number of vertebrae that could not be visualized and improving the measurement of height loss of the vertebrae). A limiting factor in the analysis of published data is the general scarcity provided on VFA methodology, particularly radiation dosages, which may have a significant impact on the quality of obtained images. In addition, studies included in our systematic review and meta-analysis were published over a more than 15-year timeframe, spanning the years 2000 to 2016 and the improvement in hardware and software of VFA technology may have potentially influenced the outcomes. The contribution of different technologies to discrepancies in the identification of vertebral fractures has been addressed in a study comparing Lunar Prodigy and Lunar iDXA densitometers, which demonstrated that iDXA had a better performance record for visualization, and thus evaluation, of vertebrae for fractures than the Prodigy densitometer (33). So far, there have been no studies comparing VFA performance between single energy and dual energy x-ray devices. The discrepancy in results of vertebral fracture assessment using VFA compared to conventional radiology in our study is in contrast to the concordance of results of assessments between radiology and VFA in the majority of studies reported in the systematic review and used in the meta-analysis. This difference could have been influenced by the different methodology used between studies. Quantitative assessment was thus used to evaluate VFA images in our study compared to the use of Genant's semi-quantitative assessment in the vast majority of studies included in the systematic review and meta-analysis.

In conclusion, from our meta-analysis findings of published data demonstrate adequate performance parameters of VFA in studies designed for patients at risk for osteoporosis, although a limitation was the very broad range of prevalent vertebral

fractures (6.9%-100%) and age (23–96 years) which may have influenced study outcomes. The data of our FLS study were in contrast with the numbers of the meta-analysis. The precise cause of the underperformance of VFA in our center is currently being investigated. Our findings suggest that caution should be advocated with the interpretation of VFA data and that centers should check the performance of their VFA device against conventional radiography of the spine before exclusively relying on this tool in the identification of vertebral fractures.

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Supplemental Table 1. Risk of bias assessment

Author	Inclusion of patients	Analysis of vertebral fracture grade	Description of vertebral fractures	Blinding of examiner
Bazzocchi	Adequate	Adequate for PV Not adequate for PP	Adequate	Adequate
Binkley	Not adequate	Adequate for PV Not reported for PP	Adequate	N/A
Chapurlat	Not adequate	Not adequate for PV Adequate for PP	Adequate	Adequate
Damiano	Not reported	Adequate for PV Not adequate for PP	Adequate	Adequate
Deleskog	Not adequate	Adequate for PV Not adequate for PP	Adequate	Adequate
Diacinti (iDXA)	Adequate	Not adequate	Adequate	N/A
Diacinti	Adequate	Not adequate	Adequate	N/A
Ferrar 2000	Not reported	Not adequate	Adequate	Not reported
Ferrar 2008	Not reported	Adequate	Adequate	Adequate
Fuerst	Not reported	Adequate	Adequate	Adequate
Hospers	Not reported	Adequate for PV Not adequate for PP	Adequate	Adequate
Mazzaferro	Not adequate	Not adequate	Adequate	N/A
Rea	Not adequate	Adequate for PV Not adequate for PP	Not adequate	N/A
Rud	Adequate	Not adequate for PV Adequate for PP	Adequate	Adequate
Schousboe	Not adequate	Adequate	Adequate	Adequate
Vokes	Not adequate	Not adequate for PV Adequate for PP	Adequate	Adequate

PV per vertebra; PP per person

N/A VFA and radiographs were evaluated by independent observers

SUPPLEMENTAL DATA

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((("vertebral fracture assessment"[all fields] OR "vertebral assessment"[all fields] OR "VFA"[all fields] OR (("assessment"[ti] OR "assessing"[ti] OR assess*[ti]) AND ("Spinal Fractures"[Majr] OR "vertebral fracture"[ti] OR "vertebral fractures"[ti] OR "spinal fracture"[ti] OR "spinal fractures"[ti] OR "spine fracture"[ti] OR "spine fractures"[ti] OR ((spine[ti] OR spinal[ti] OR spines[ti] OR vertebral[ti] OR vertebrae[ti] OR vertebr*[ti]) AND (fracture[ti] OR fractures[ti] OR fractur*[ti]))))) AND ("Densitometry"[mesh] OR "dual X-ray absorptiometry"[all fields] OR "DXA"[all fields] OR "DEXA"[all fields] OR "densitometry"[all fields] OR densitometr*[all fields] OR "absorptiometry"[all fields] OR absorptiometr*[all fields]) AND ("Radiography"[Mesh:NoExp] OR "radiography"[Subheading] OR "radiography"[all fields] OR radiograph*[all fields] OR "Xray"[all fields] OR "Xrays"[all fields] OR "X-ray"[all fields] OR "X-rays"[all fields] OR "roentgenography"[all fields] OR "rontgenography"[all fields] OR "roentgen"[all fields] OR "rontgen"[all fields] OR roentgen*[all fields] OR rontgen*[all fields]) AND ("Comparative Study"[Publication Type] OR comparative[all fields] OR compared[all fields] OR comparison[all fields] OR compar*[all fields] OR "Reproducibility of Results"[mesh] OR "Sensitivity and Specificity"[mesh] OR "Limit of Detection"[all fields] OR "ROC Curve"[all fields] OR "Signal-To-Noise Ratio"[all fields] OR "Sensitivity"[all fields] OR "Specificity"[all fields] OR "Reproducibility of Results"[all fields] OR "Dimensional Measurement Accuracy"[all fields] OR "Reproducibility"[all fields] OR "Reliability"[all fields] OR "Validity"[all fields])) OR ((("vertebral fracture assessment"[all fields] OR "vertebral assessment"[all fields] OR "VFA"[all fields] OR (("assessment"[all fields] OR "assessing"[all fields] OR assess*[all fields]) AND ("Spinal Fractures"[Mesh] OR "vertebral fracture"[all fields] OR "vertebral fractures"[all fields] OR "spinal fracture"[all fields] OR "spinal fractures"[all fields] OR "spine fracture"[all fields] OR "spine fractures"[all fields] OR ((spine[ti] OR spinal[ti] OR spines[ti] OR vertebral[ti] OR vertebrae[ti] OR vertebr*[ti]) AND (fracture[ti] OR fractures[ti] OR fractur*[ti]))))) AND ("Densitometry"[majr] OR "dual X-ray absorptiometry"[ti] OR "DXA"[ti] OR "DEXA"[ti] OR "densitometry"[ti] OR densitometr*[ti] OR "absorptiometry"[ti] OR absorptiometr*[ti]) AND ("Radiography"[Mesh:NoExp] OR "radiography"[Subheading] OR "radiography"[all fields] OR radiograph*[all fields] OR "Xray"[all fields] OR "Xrays"[all fields] OR "X-ray"[all fields] OR "X-rays"[all fields] OR "roentgenography"[all fields] OR "rontgenography"[all fields] OR "roentgen"[all fields] OR "rontgen"[all fields] OR roentgen*[all fields] OR rontgen*[all fields]) AND ("Comparative Study"[Publication Type] OR comparative[all fields] OR compared[all fields] OR comparison[all fields] OR compar*[all fields] OR "Reproducibility of Results"[mesh] OR "Sensitivity and Specificity"[mesh] OR "Limit of Detection"[all fields] OR "ROC Curve"[all fields] OR "Signal-To-Noise Ratio"[all fields] OR "Sensitivity"[all fields] OR

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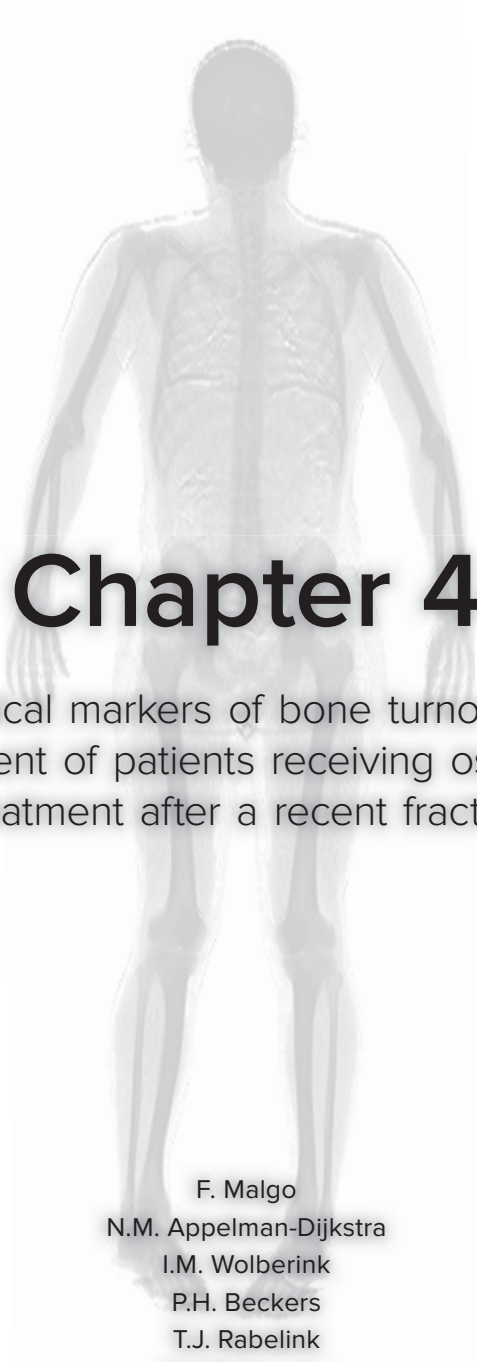
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sitometr* OR "absorptiometry" OR absorptiometr*) AND TS=(radiography OR bone radiography OR "radiography" OR radiograph* OR "Xray" OR "Xrays" OR "X-ray" OR "X-rays" OR "roentgenography" OR "rontgenography" OR "roentgen" OR "rontgen" OR roentgen* OR rontgen*) AND TS=("Comparative Study" OR comparative OR compared OR comparison OR compar* OR measurement precision OR Reproducibility OR "Sensitivity and Specificity" OR "Limit of Detection" OR "ROC Curve" OR "Signal-To-Noise Ratio" OR "Sensitivity" OR "Specificity" OR "Reproducibility of Results" OR "Dimensional Measurement Accuracy" OR "Reproducibility" OR "Reliability" OR "Validity")) OR (TS=("vertebral fracture assessment" OR "vertebral assessment" OR "VFA") AND TS=("Densitometry" OR "dual X-ray absorptiometry" OR "DXA" OR "DEXA" OR "densitometry" OR densitometr* OR "absorptiometry" OR absorptiometr*) AND TS=(radiography OR bone radiography OR "radiography" OR radiograph* OR "Xray" OR "Xrays" OR "X-ray" OR "X-rays" OR "roentgenography" OR "rontgenography" OR "roentgen" OR "rontgen" OR roentgen* OR rontgen*) AND TS=("Comparative Study" OR comparative OR compared OR comparison OR compar* OR measurement precision OR Reproducibility OR "Sensitivity and Specificity" OR "Limit of Detection" OR "ROC Curve" OR "Signal-To-Noise Ratio" OR "Sensitivity" OR "Specificity" OR "Reproducibility of Results" OR "Dimensional Measurement Accuracy" OR "Reproducibility" OR "Reliability" OR "Validity"))



Chapter 4

Biochemical markers of bone turnover in the management of patients receiving osteoporosis treatment after a recent fracture

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P.H. Beckers
T.J. Rabelink
N.A.T. Hamdy

Submitted

ABSTRACT

Summary:

This study evaluated the changes in BTMs and their relationship with BMD measurements. We found that changes in BTMs were related with lumbar spine BMD early in the treatment with anti-osteoporosis agents.

Introduction:

Bone turnover markers (BTMs) can be easily measured in serum and have the potential to be used as a tool to monitor treatment.

Methods:

A prospective study to report the longitudinal pattern of P1NP and Ctx after 3 and 12 months of antiresorptive treatment and to evaluate their relationship with Bone Mineral Density (BMD) values after 12 months of treatment in patients attending a Fracture Liaison Service who have recently sustained a fracture.

Results:

127 patients were included in the final analysis. One had a normal BMD, 43 had osteopenia and 83 patients had osteoporosis. Changes in P1NP and Ctx after 12 months of treatment were significantly inversely related with changes in lumbar spine BMD after 12 months ($B = 0.043$, $p = 0.003$; $B = 0.066$, $p = 0.020$, respectively). Baseline P1NP was significantly correlated with the change in BMD at the lumbar spine ($B = 0.018$, $p = 0.014$).

Conclusion:

Our data suggest that early changes in BTMs or baseline measurements of these markers are predictive for the outcome of treatment with anti-osteoporosis drugs. Whether these markers could be used to predict the risk for fractures remains to be established.

INTRODUCTION

Osteoporosis is a systemic bone disease characterized by low bone mass and microarchitectural deterioration of bone tissue leading to increased bone fragility and fracture risk (1). Projections for Europe suggest that the number of fractures due to osteoporosis will increase by 28% to an estimated 4.5 million in 2025, resulting in a significant increase in personal, societal and economic burdens with consequent increase in Disability Adjusted Life Years (DALY) and general quality of life (2). Based on these alarming predictions, recent guidelines have focused on secondary prevention of fractures by advocating the establishment of Fracture Liaison Services (FLSs), which essentially captures patients at high risk for fractures by implementing a multidisciplinary care trajectory for screening of all patients, male or female aged 50 years or older who recently sustained a fracture for the presence of osteoporosis using Bone Mineral Density (BMD) measurements (3). Treatment using anti-osteoporosis drugs shown to be effective in decreasing fracture risk forms an intrinsic part of the care trajectory for patients considered at high risk for new fractures (4–6).

Bone turnover markers (BTMs) can be easily measured in serum collected at baseline and at variable intervals after start of treatment. BTMs recommended for use in osteoporosis include the bone formation marker serum procollagen 1 N-terminal extension peptide (P1NP), and the bone resorption marker serum C-terminal cross-linked telopeptide of type I collagen (CTX), both having been found to be the markers of choice in osteoporosis (7). In the management of osteoporosis, BTMs have been shown to decrease within weeks, whereas changes in BMD are slower, only observed after at least a year of treatment with the use of antiresorptive agents. Moreover, it has been shown that a decrease in BTMs is correlated with an increase in BMD and with anti-fracture efficacy (8, 9). Based on these data, one may suggest that BTMs have the potential to be used as a tool to monitor treatment and may predict treatment outcome. However, the experience on BTMs outside clinical trials remains scarce. In addition, BTMs are elevated for several months in the healing process after a fracture, which may complicate the interpretation of these markers.

In this study, we aimed to describe the response of BTMs after 3 and 12 months of antiresorptive treatment and their relationship with BMD measurements after 12 months of treatment in patients attending a FLS who have recently sustained a fracture.

PATIENTS AND METHODS

Study design and population

All patients aged 50 years or older who had sustained a fracture from June 2012 to June 2014 for which they presented at the Emergency Room of the Leiden University Medical Center (LUMC) and were subsequently referred to our Fracture Liaison Service (FLS) of the LUMC were considered for inclusion in the study if started on osteoporosis treatment using oral bisphosphonates (BPor), intravenous bisphosphonates (BPiv) or denosumab (Dmab) as required. Patients with fractures of skull, hands or feet were excluded. Details of the methodology of the study are described in a previous publication from our Center (10).

The following inclusion criteria were additionally used in this particular study: osteoporosis treatment had to have been started within 3 months of baseline evaluation, and data on P1NP and BMD had to be available at baseline and 12 to 15 months after start of treatment. Patients who were already using osteoporosis treatment or those with severe liver insufficiency or CKD grade IV-V were excluded from the study.

The treatment regimens used in our FLS protocol are in keeping with Dutch national guidelines for the management of osteoporosis (11). Choice of treatment was left at the discretion of the treating physician after taking patient preference for treatment in consideration. Follow-up visits were planned at 12 months after start of treatment for all patients and additionally at 3 months for patients started on oral or intravenous bisphosphonates. The study was approved by the Medical Ethical Committee of the LUMC.

Study parameters

Data on age, gender, weight and height [from which the body mass index (BMI) was calculated], medication history including bone modifying agents and vitamin D supplementation, the date of the most recent fracture and of the first visit to the FLS, and a full medical history including detailed fracture history, family history of osteoporosis, history of parental hip fractures, age at menopause, dietary calcium intake, alcohol use and smoking habits were collected at baseline. Data on the reason and time of substitution of one type of osteoporosis drug for another or time and reason for discontinuation of treatment were also recorded if applicable.

Biochemical parameters

Serum obtained from blood samples collected at baseline and 3 and 12 months after start of treatment was measured for calcium, albumin, inorganic phosphate, alkaline phosphatase, creatinine, PTH, 25-OH vitamin D, P1NP and CTX.

Bone Mineral Density

BMD was measured at the lumbar spine (L1-L4) and at the left and right femoral neck at baseline and 12 months after start of treatment using Dual Energy X-ray Absorptiometry (DXA) with a Hologic QDR Discovery A scanner (Hologic, Bedford, MA, USA). T-scores were calculated using reference values from the Third National Health and Nutrition Survey (NHANES III) compatible with those of the Dutch population. Using WHO criteria, osteoporosis was defined as a BMD ≥ 2.5 standard deviation (SD) below the average value for young healthy women, expressed as a T-score of ≤ -2.5 . Osteopenia was defined as a BMD $> 1SD$ but $< 2.5 SD$ below the young adult mean (T-score between -2.5 and -1) and normal BMD was defined as a T-score ≥ -1.0 .

Statistical analysis

All analyses were performed using SPSS software for Windows (Version 23.0 Chicago, IL, USA). Between-group differences in baseline characteristics were assessed using an ANOVA test for numerical data and a Chi-squared test for categorical data. Spearman's correlation coefficient was used to analyse the correlation between bone turnover markers and BMD at baseline and after 12 months of treatment, after performing Kolmogorov-Smirnoff tests, which showed not normally distributed BTM-values. Linear regression analysis was performed using changes in BTMs between baseline (T₀) and 12 months (T₁₂) and baseline (T₀) and 3 months (T₃), and the differences in DXA measurements between T₀ and T₁₂. A *p*-value < 0.05 was considered to be statistically significant.

RESULTS

Patient characteristics

252 patients, 197 women and 55 men aged 50 years or older, who were referred to the FLS after sustaining a recent fracture, were found to be at high fracture risk and were eligible for receiving osteoporosis treatment. During the year of follow-up, 7 patients had discontinued therapy within 3 months, and 90 had no follow-up BMD at 1 year (4 had died, 7 had comorbidity that prevented them from attending follow-up visits, 32 were referred back to their general practitioner, 4 were referred to another specialist, 19 had follow-up BMD measurements > 15 months after start of treatment and 24 were lost to follow-up for unclear reasons), In addition, 23 patients had no available data on P1NP at baseline and another 5 no available data at 1-year of follow-up. A full set of BMD and P1NP data at baseline and 1-year follow up was thus available in 127 patients who were included in the final analysis of data; Figure 1.

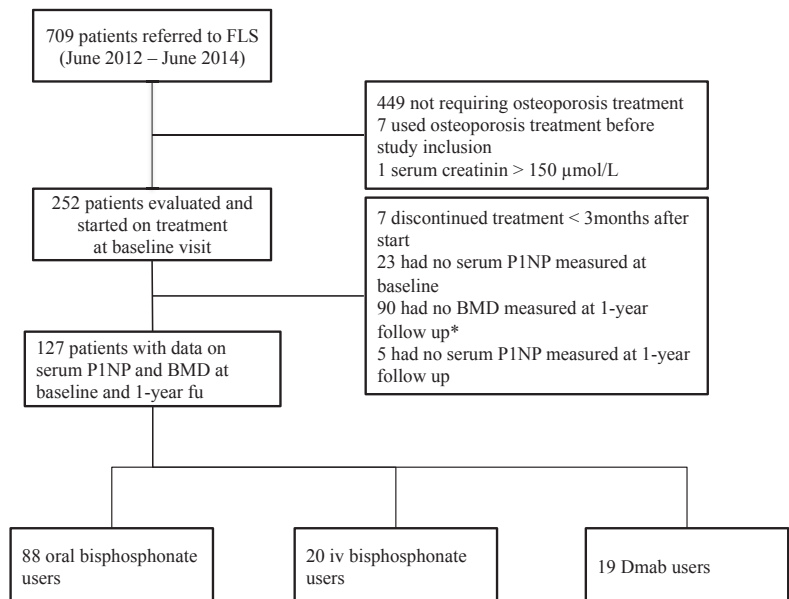


Figure 1

Patient enrolment

FLS Fracture Liaison Service; BMD Bone Mineral Density; fu follow-up; in intravenous; Dmab Denosumab

*

- 4 had died,
- 7 had comorbidity
- 32 were referred back to their general practitioner
- 4 were referred to another specialist
- 19 had follow-up BMD measurements > 15 months after start of treatment
- 24 were lost to follow-up for unclear reasons

These were 32 men and 95 women with a mean age of 67.0 years (range 50.0 – 89.6 years) and BMI of $25.3 \pm 3.8 \text{ kg/m}^2$. The majority of patients had sustained a fracture of the distal radius [$n = 37$ (29%)], 23 (18%) of the proximal humerus, 18 (14%) of the hip and 14 (11%) of one or more vertebrae. Mean time between the recently sustained fracture and baseline visit to the FLS was 3.0 ± 2.6 months; Table 1.

At baseline, 88 patients started oral BP therapy (BPor), 20 intravenous BP therapy (BPiv) and 19 Denosumab (Dmab). During the year of follow-up, 6 patients were switched from BPor to BPiv or Dmab at 3 months and 1 was switched at 6 months. Of these 7 patients, two switched treatment to BPiv and five to Dmab. Nine patients sustained a new fracture after start of anti-osteoporosis treatment.

Baseline values of Bone Mineral Density and bone turnover markers

Eighty-three patients had osteoporosis, forty-three had osteopenia and one had a normal BMD. Mean BMD was $0.84 \pm 0.13 \text{ g/cm}^2$ at the lumbar spine and $0.63 \pm 0.09 \text{ g/cm}^2$

Table 1. Baseline characteristics of patients

	Total (n = 127)	BPor (n = 88)	BPiv (n = 20)	Dmab (n = 19)
Age (years)	67.0 ± 9.3	70.4 ± 10.4	70.6 ± 10.8	73.9 ± 9.5
Male/female (%)	32/95 (25%/75%)	23/65 (26%/74%)	7/13 (35%/65%)	2/17 (11%/89%)
BMI (kg/m ²)	25.3 ± 3.8	25.4 ± 4.0	24.8 ± 3.9	24.9 ± 4.3
Previous Fracture (%)	52 (41%)	86 (49%)	19 (49%)	12 (32%)
FRAX score major fracture	13.0 ± 0.7	13.0 ± 0.6	18.0 ± 1.8	15.5 ± 0.9
FRAX score hip fracture	4.0 ± 0.6	5.0 ± 0.5	8.9 ± 1.6	5.8 ± 0.8
Laboratory data:				
Creatinine (μmol/L)	72.4 ± 15.3	72.1 ± 20.5	67.2 ± 13.7	80.1 ± 29.5
Calcium (mmol/L)	2.35 ± 0.09	2.36 ± 0.09	2.35 ± 0.13	2.36 ± 0.08
ALP (U/L)	101.2 ± 41.4	103.7 ± 43.7	99.1 ± 32.6	99.8 ± 34.2
PTH (pmol/L)	4.1 ± 2.2	4.2 ± 3.1	4.6 ± 1.9	4.7 ± 2.9
25-OH D (nmol/L)	66.7 ± 28.0	63.4 ± 28.1	72.0 ± 34.2	57.5 ± 22.8
P1NP (ng/mL)	75.8 ± 37.1	83.7 ± 58.4	59.1 ± 26.4	80.7 ± 51.5
CTX (ng/mL)	0.388 ± 0.176	0.406 ± 0.199	0.312 ± 0.145	0.453 ± 0.259
DXA measurements:				
LS BMD (g/cm ²)	0.84 ± 0.13	0.86 ± 0.14	0.83 ± 0.17	0.87 ± 0.16
FN BMD (g/cm ²)	0.63 ± 0.09	0.63 ± 0.10	0.60 ± 0.10	0.61 ± 0.08

BMI Body Mass Index; ALP alkaline phosphatase; PTH parathyroid hormone; DXA Dual Energy X-Ray Absorptiometry; LS Lumbar Spine; FN Femoral Neck; BMD Bone Mineral Density

at the femoral neck. Mean serum level of P1NP was 75.7 ng/ml (range 23–240 ng/ml) at baseline. There was no difference in P1NP values between patients with osteoporosis, osteopenia or normal BMD (76.1 ± 32.8 , 75.3 ± 45.0 , 75.0 ; $p = 0.464$). Mean P1NP did not correlate with mean BMD at the lumbar spine or at the femoral neck ($r = -0.066$, $p = 0.461$; $r = -0.098$, $p = 0.281$, respectively).

Data on Ctx levels at baseline were available in 90 patients and mean serum level was 0.387 ng/ml (range 0.120–0.940 ng/ml). There was no difference in Ctx values between patients with osteoporosis, osteopenia or normal BMD (0.400 ± 0.184 , 0.349 ± 0.137 , 0.904 ; $p = 0.121$). Mean Ctx did not correlate with mean BMD at the lumbar spine or at the femoral neck ($r = -0.135$, $p = 0.206$; $r = -0.043$, $p = 0.691$, respectively).

Follow-up changes in Bone Mineral Density and bone turnover markers after 12 months

BMD values increased by 4.4% from 0.84 ± 0.13 g/cm² to 0.88 ± 0.13 g/cm² at the lumbar spine, and by 1.6% from 0.63 ± 0.09 g/cm² to 0.64 ± 0.09 g/cm² at the femoral

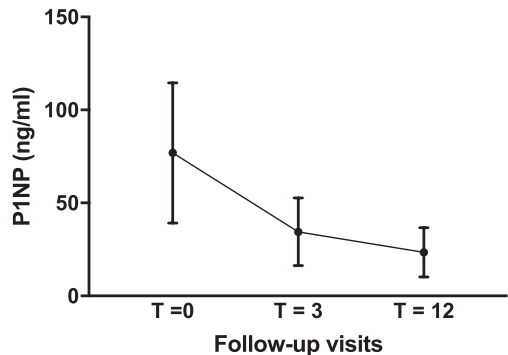


Figure 2
Change in P1NP levels after 3 and 12 months of treatment

neck. Mean P1NP decreased by 64% from 75.7 ng/ml to a mean of 23.3 ng/ml (range 7–105 ng/ml) after one year of treatment. In 82 of the 127 patients, P1NP was also measured 3 months after start of treatment. At this time-point, serum levels of P1NP decreased by 51% to 34.5 ± 18.2 ng/ml compared to baseline and by another 23% to 23.5 ± 13.3 ng/ml after 12 months of treatment; Figure 2.

Mean Ctx decreased by 58% from 0.387 ng/ml to a mean of 0.138 ng/ml (range 0.046–0.416 ng/ml) after one year of treatment.

Predictive value of bone turnover markers for changes in Bone Mineral Density

The change in serum levels of P1NP between baseline and 1-year follow-up (ΔT_0 -T12) was predictive for (ΔT_0 -T12) BMD at the lumbar spine, with a decrease in P1NP being predictive for a significant increase in lumbar spine BMD ($B = 0.046$, $p = 0.001$), also after adjusting for time after the fracture ($B = 0.043$, $p = 0.003$); Figure 3. Changes in

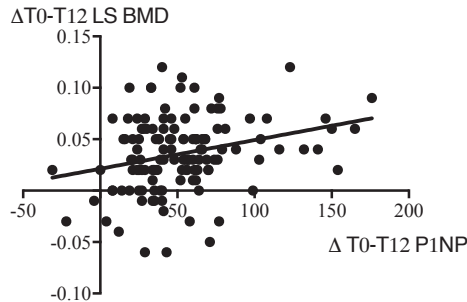


Figure 3
Significant relationship between the difference in P1NP at baseline and 12 months follow-up and the difference in lumbar spine BMD at baseline and 12 months follow-up

serum P1NP levels after 1 year were not, however, predictive for changes in BMD at the femoral neck ($B = -0.010$, $p = 0.398$).

The change in P1NP concentrations after 3 months of treatment was predictive for (ΔT_0 -T12) BMD at the lumbar spine ($B = 0.035$, $p = 0.045$) but not for (ΔT_0 -T12) BMD at the femoral neck ($B = -0.016$, $p = 0.345$). (ΔT_0 -T3) P1NP after 3 months was not predictive for (ΔT_0 -T12) BMD at the lumbar spine after adjusting for time after fracture ($B = 0.028$, $p = 0.124$).

(ΔT_0 -T12) Ctx was predictive for (ΔT_0 -T12) BMD at the lumbar spine ($B = 0.071$, $p = 0.011$), also after adjusting for time after the fracture ($B = 0.066$, $p = 0.020$), but was not predictive for changes in BMD at the femoral neck ($B = -0.005$, $p = 0.840$).

We could further evaluate whether individual serum levels of P1NP at baseline were predictive for changes in BMD after 1 year of treatment in 132 patients in whom data were available for BMD at baseline and follow up and for P1NP at baseline. Higher baseline P1NP was found to be predictive for a positive change in BMD at the lumbar spine ($B = 0.021$, $p = 0.003$), also after adjustment for time after fracture ($B = 0.018$, $p = 0.014$). Baseline P1NP could not predict the change in BMD at the femoral neck ($B = -0.10$, $p = 0.069$).

Baseline Ctx levels were not predictive for changes in BMD at the lumbar spine ($B = 0.039$, $p = 0.240$) or femoral neck ($B = -0.022$, $p = 0.420$).

DISCUSSION

Our data from this study demonstrate a relationship between changes in BMD at the lumbar spine and changes in P1NP and CTx levels after 12 months of starting osteoporosis treatment after a recent fracture, also after adjustment for time between fracture and the initial FLS evaluation. Changes in P1NP levels after 3 months were related with (ΔT_0 -T12) BMD at the lumbar spine, but this did not reach significance after adjustment for time between fracture and the first FLS visit. We also observed a positive relationship between baseline P1NP before start of treatment and (ΔT_0 -T12) BMD at the lumbar spine, also after adjustment of time after fracture.

In the management of osteoporosis, bone turnover markers have been shown to decrease within weeks after start of antiresorptive agents, whereas changes in BMD are slower, only observed after at least a year of treatment (4, 5, 12, 13). Although these observations suggest that bone turnover markers may be an attractive tool for the monitoring of treatment of osteoporosis using bone modifying agents, there is to date no consensus on the use of these markers in the individual patient in daily clinical practice.

In a position paper that focuses on the value of using bone turnover markers in monitoring osteoporosis treatment, Vasikaran and colleagues have described a number of factors that might affect the pattern of response of these markers, such as the nature, dose and route of administration of the given agent (7). In order to use BTMs in the monitoring of osteoporosis treatment, baseline measurements and follow-up measurements after a specified interval are required. The position paper does not provide an advice for the time interval that should be used but refers to a number of studies that measured BTMs after 3, 6 and 12 months of treatment and found that the reduction in BTMs was inversely related with the reduction in the risk of fractures. Our study describes data on BTMs measured at baseline and after 3 and 12 months of treatment with antiresorptives that were found to be related with BMD outcome.

Interestingly, there was also a relationship between baseline levels of P1NP and the change in lumbar spine BMD after 12 months although BTMs measured at baseline are heavily affected by the effect of the recently sustained fracture. In keeping with these findings is the observation that postmenopausal women with high BTMs at baseline have a greater effect on anti-fracture efficacy after start of treatment (14–16). However, patients with high P1NP levels at baseline in our study might also have had high P1NP levels before they sustained a fracture. We could not test this hypothesis as we lack data on pre-fracture P1NP measurements.

Our study has strengths as well as limitations. One of its strengths is that data was collected in consecutive patients who have recently sustained a fracture, were aged 50 year or older and were referred to our FLS. The Leiden University Medical Center is a tertiary center but its FLS serves a large regional treatment area. Osteoporosis treatment was predominantly given in the form of oral bisphosphonates, which again is representative of daily clinical practice. We only included patients with available data on BMD and BTMs at 1-year follow-up so that we could evaluate whether these markers could reflect a change in bone mass. This may have also limited our study as it could have led to a selection bias as only those patients with adequate adherence to treatment may have been included.

In conclusion, our study demonstrates that a decrease in P1NP and Ctx levels at 12 months after starting anti-resorptive agents and higher baseline P1NP levels are predictive for a significant increase in BMD at the lumbar spine after 12 months of follow-up. These findings suggest that changes in bone turnover markers or measurements of these markers at baseline could be used to predict the effect of treatment with anti-osteoporosis drugs. Large follow-up studies are required to establish the relationship between the risk of fracture and the change in bone turnover markers or baseline measurements.

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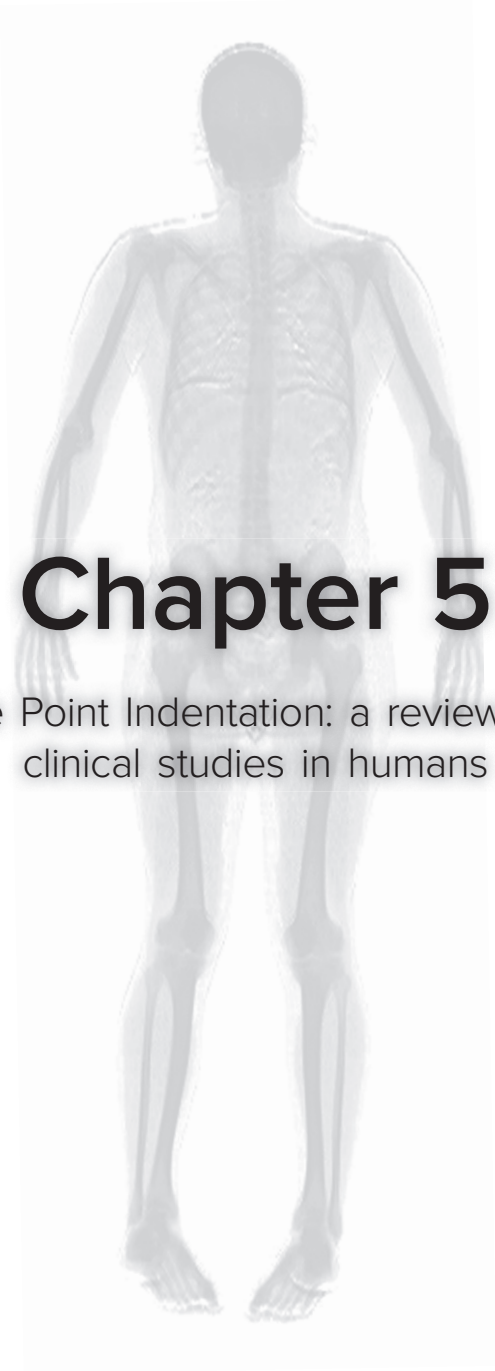
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Part 2

Impact microindentation
in the evaluation of bone fragility



Chapter 5

Reference Point Indentation: a review of *in vivo* clinical studies in humans

ABSTRACT

Impact microindentation (IMI) is a novel technique, which has been introduced in the clinic for the evaluation of tissue-level properties of cortical bone. The number of studies addressing the value of this technique in the management of osteoporosis has been steadily increasing over the past decade. This chapter focuses on a review of published clinical studies using the IMI technique *in vivo* in humans with special emphasis on the four studies which have so far explored the relationship between IMI-derived Bone Material Strength index (BMSi) and fracture.

Available data from these studies provide evidence for the value of IMI in differentiating between patients with increased fracture risk and control patients. However, a main limitation identified in the interpretation of IMI data is the high variability in BMSi in patients as well as control populations between studies. This observed variability is likely to be due to the absence of a standard protocol for performing the procedure, leading to variable study protocols being used by different centers. Notwithstanding, data gathered so far from our studies and that of others suggest that IMI represents a valuable additive tool in the evaluation of elements of bone fragility which are not fully captured by currently used methods such as DXA.

INTRODUCTION

Bone fragility is a complex concept and its evaluation remains a challenge in clinical practice. The assessment of bone fragility has to date mainly focussed on the evaluation of clinical risk factors for increased bone fragility together with quantitative evaluation of bone mass by Dual Energy X-ray Absorptiometry (DXA) (1–5). Bone Mineral Density (BMD) measurements have been performed in the clinic for over three decades and experience with their use is substantial. However, evidence has been accumulating for limitations of this technique as it has now been demonstrated that only one third of an individuals' fracture risk can be explained by BMD values (1). Other available tools to assess bone fragility include High Resolution peripheral Quantitative Computed Tomography (HRpQCT) to assess bone structure, bone biopsies to assess bone histomorphometry and nano-indentation to assess material properties of bone (6, 7). However, these methods are associated with either high radiation exposure or with the use of invasive and time-consuming procedures so that their use has so far been largely restricted to research settings. Material properties of bone significantly contribute to bone fragility but the study of these parameters is difficult. Traditional mechanical tests such as bending or compression techniques, considered as the golden standard for evaluating material properties of bone, cannot be performed *in vivo*.

Reference Point Indentation (RPI) has been introduced just over a decade ago for the evaluation of *in vivo* tissue-level properties of cortical bone (4, 5). In contrast to other indentation techniques, RPI is designed to be used without surgically exposing the bone surface and does not require the use of an optic microscope to register the deformation of the indentations (8).

The principle of RPI is based on the hypothesis that a fracture is precipitated by the separation of mineralized collagen microfibers, leading to microcracks (9). These microcracks are similar to those observed in fractured bone, so that it has been hypothesized that RPI measurements may be of value in assessing bone's ability to resist fracture or in other words in reflecting bone fragility. Since the introduction of the RPI technique in 2006, experience has been accumulating with the use of this technique with increasing numbers of patients being tested. An adaption of the original device has been developed and applied and a standard operating procedure has been recently published (10, 11).

This Review Chapter primarily focuses on published clinical studies using the Reference Point Indentation technique *in vivo* in humans, and summarizes reported data and clinical implications regarding the use of this technique in the evaluation of tissue level properties of cortical bone and possibly beyond overall bone fragility.

THE REFERENCE POINT INDENTATION TECHNIQUE

Two Reference Point Indentation devices are currently available, namely the Osteoprobe for *in vivo* use in humans and large animals, and the Biodent, which is mainly used for basic research studies although the first two human studies were performed using this device. To avoid confusion with the interpretation of data, a unified nomenclature has been proposed in a recent publication: cyclic reference point microindentation (CMI) is performed using the Biodent device and impact microindentation (IMI) is performed using the Osteoprobe RUO device (11).

Although the main principle of indenting the bone surface holds for both devices, there are significant differences between the two tools. Firstly, the Biodent makes use of a cyclic loading system and the Osteoprobe of single impact loading. Secondly, Biodent drives the test probe into the bone with a load from 2 to 10 Newton with an adjustable number of cycles (up to 20), whereas the Osteoprobe drives the test probe into the bone with a 30 Newton load, after a pre-load of 10 Newton to stabilize the test probe. Thirdly, several parameters are acquired with the Biodent using the force-distance curves, including the total Indentation Distance Increase, Indentation Distance Increase (IDI), creep Indentation Distance, loading and unloading slope and the average Energy Dissipation. In contrast, the Osteoprobe has only one outcome parameter: Bone Material Strength index (BMSi), which is calculated using the indentation distance increase (IDI) from the test probe during the load application. After the measurements on the bone tissue, five measurements are performed on a calibration phantom consisting of polymethylmetacrylate (PMMA). BMSi is defined as 100 times the harmonic mean of IDI from impact into the PMMA divided by the average IDI from impact into bone tissue (10, 11). Fourthly, the Osteoprobe differs from the Biodent in that it lacks a reference probe but uses an inertial reference point located at the point from the test probe just before the impact is activated, thereby eliminating the need to scrape the periosteum.

A further practical difference between the two devices is that measurements using the Biodent need to be performed using a fixed device mounted over the patient whereas the Osteoprobe is a handheld device, which facilitates its use in clinical practice, particularly in an outpatient setting.

Clinical studies using the Biodent

Diez-Perez and colleagues were the first to report CMI data in 27 postmenopausal women who had sustained osteoporotic fractures (25 hip, 2 vertebra) and 8 controls (9). Their data showed significantly greater indentation distances in fracture patients compared to controls, with the caveat that total hip BMD was significantly higher in the latter group. CMI measurements were also performed on cadaveric bone samples of

the tibia of 5 human donors (age range 17 – 74 years; 2 male, 3 female). Mechanical tests performed to measure crack growth toughness, showed a significant inverse relationship between IDI and crack growth toughness. Based on these data, the researchers raised the hypothesis that microindentation may induce microcracks on the bone surface and may in turn reflect bone fragility.

This publication was followed by another study using the Biodent in 6 patients with atypical femoral fractures, 38 patients with hip fractures, 6 patients without incident fractures but using long-term bisphosphonates, and 20 non-fracture controls (12). In keeping with the initial CMI study, patients with hip fractures had significantly greater IDI compared to controls without fractures. In addition, IDI was greater in AFF patients than in controls, but was comparable to that of hip fracture patients suggesting that AFF patients and conventional hip fracture patients have similar tissue-level properties of bone. IDI of long-term bisphosphonate users with no incident fracture during treatment was comparable with IDI of controls, suggesting similar tissue-level properties of bone between these two groups.

These two studies were the first to provide information on bone material properties in patients with osteoporotic fractures and AFF, as well as experience with the use of the technique of microindentation. Several adaptations and improvements were subsequently applied to the Osteoprobe instrument, which has been further solely used for *in vivo* clinical studies in humans (Table 1).

Clinical studies using the Osteoprobe

Fifteen studies have been so far published on the *in vivo* use of the IMI technique in humans. Most of these studies included patient groups with an increased risk for fracture not fully explained by BMD values. An interesting study from Sweden evaluated the association between BMSi, micro architectural parameters of bone and subcutaneous fat at the tibia in 202 elderly women with no specific underlying pathology (13). The investigators found an inverse relationship between BMSi and the amount of subcutaneous fat, whole body fat mass and BMI. In addition, BMSi was associated with cortical porosity and cortical volumetric BMD at the distal tibia. These findings suggest a negative influence of adipose tissue on bone strength.

Effect of type 2 diabetes mellitus on BMSi measurements

The first published IMI study was from the United States and evaluated Bone Material Strength index in 30 postmenopausal women with type 2 diabetes mellitus (T2DM) compared to 30 healthy controls (14). In this study, the authors observed that BMSi was significantly lower in patients with T2DM compared to controls, also after adjustment for multiple variables potentially confounding according to the authors (e.g. BMI, hy-

Table 1. Clinical studies using impact microindentation

Subjects (m/f)			Age	FN BMD	BMSi	Report of BMSi
ActiveLife						
Bridges	—	—	—	—	—	
2012						
Randall	—	—	—	—	—	
2013						
Peer-reviewed In humans						
Farr	T2DM	30 (0/30)	65.4 ± 7.7	0.93 ± 0.03	77.2 ± 1.6	Mean ± SEM
2014 (US)	Controls	30 (0/30)	65.7 ± 8.8	0.94 ± 0.03	85.27 ± 1.6	
Malgo	Fracture	63 (24/39)	62.6 ± 9.6	0.67 ± 0.09	79.4 ± 0.7	Mean ± SEM
2015 (NL)	No fracture	27 (13/14)	57.1 ± 9.5	0.69 ± 0.08	83.6 ± 1.1	
Mellibovsky	Ca/D3	19 (11/8)	55.3 ± 17.9	0.83 ± 0.13	81.6 (74.3 - 86.9)	Median (IQR)
2015 (ES)	RSN	14 (10/4)	66.1 ± 17.0	0.75 ± 0.14	81.1 (75.6 - 89.6)	
	Dmab	14 (5/9)	58.9 ± 12.8	0.72 ± 0.15	76.2 (72.0 - 84.9)	
	TPTD	5 (1/4)	69.8 ± 8.0	0.62 ± 0.12	70.0 (64.0 - 72.6)	
Duarte Sosa	Norwegian	42 (0/42)	46.3 ± 13.6	1.03 ± 0.10	77.0 ± 7.1	Mean ± SD
2015 (NO)	Spanish	46 (0/46)	46.7 ± 15.4	0.83 ± 0.12	80.7 ± 7.8	
Rudang	Fracture	117 (0/117)	78.3 ± 1.1	0.64 ± 0.10	76.1 ± 7.4	Mean ± SD
2016 (SE)	No fracture	63 (0/63)	78.2 ± 1.1	0.69 ± 0.09	75.7 ± 7.9	
Sundh *	Elderly women	202 (0/202)	—	—	—	
2016 (SE)						
Aasarod	CAG	14 (6/8)	Not reported	Not reported	82.0 ± 9.6	Mean ± SD
2016 (NO)	Controls	18 (9/11)			80.0 ± 7.0	
Furst	T2DM	16 (0/16)	65.4 ± 2.4	0.77 ± 0.03	63.7 ± 1.9	Mean ± SEM
2016 (US)	Controls	19 (0/19)	65.6 ± 1.2	0.69 ± 0.01	70.1 ± 1.9	
Guérri-	HIV	50 (35/15)	36.7	0.81 (0.77-0.88)	85.0 ± 5.4	Mean ± SD
Fernandez	Controls	35 (24/11)	33.9	0.79 (0.73-0.96)	91.0 ± 4.7	
2016 (ES)						
Duarte Sosa	Stress fracture	30 (0/30)	39.0 ± 13.9	0.92 ± 0.25	70.5 ± 8.7	Mean ± SD
2016 (NO)	No fracture	30 (0/30)	42.3 ± 9.8	1.05 ± 0.11	77.1 ± 7.2	
Perez-Saez	Renal Tx	40 (17/23)	63.8 ± 11.1	0.67 ± 0.13	79.1 ± 7.7 **	Mean ± SD
2016 (ES)	Controls	94 (20/74)	50.2 ± 16	0.78 ± 0.12	82.9 ± 7.8	
Nilsson	T2DM	51 (0/51)	Not reported	Not reported	74.6 ± 7.6	Mean ± SD
2016 (SE)	Controls	438 (438)			78.2 ± 7.5	
Malgo	Acromegaly	48 (26/22)	60.2 ± 11.0	0.84 ± 0.16	79.4 ± 0.7	Mean ± SEM
2017 (NL)	Controls	44 (22/22)	60.5 ± 8.5	0.80 ± 0.09	83.2 ± 0.7	
Duarte Sosa	Fracture	66 (0/66)	68.2 ± 9.9	Not reported	71.5 ± 7.3	Mean ± SD
2017 (NO)	Control	66 (0/66)	66.5 ± 7.9	1.02 ± 0.13	76.4 ± 6.2	
Herrera	Gaucher	16 (7/9)	51.3 ± 14.4	0.72 ± 0.17***	72.7 ± 10.0	Mean ± SD
2017 (ES)	Control	29 (5/24)	48.0 ± 12.1	0.77 ± 0.11	81.8 ± 1.4	

US United States; NL The Netherlands; ES Spain; NO Norway; SE Sweden.

FN BMD femoral neck Bone Mineral Density; BMSi Bone Material Strength index; T2DM type 2 diabetes mellitus; Ca/D3 calcium/ 25-OH vitamin D supplementation; RSN Risedronate; Dmab Denosumab; TPTD Teriparatide; CAG chronic atrophic gastritis; HIV human immunodeficiency virus; Renal Tx renal transplantation

* Sundh and Rudang reported the same patient group

** BMSi was measured in 38 renal Tx patients and 93 controls

*** Total hip BMD was reported

pertension, retinopathy). There was a relationship between BMSi and mean glycated haemoglobin level over the 10 years prior to the study, but not with current HbA1c as measured at the screening visit, or with other variables such as age, BMI or BMD.

Two further studies confirmed the association between BMSi and T2DM. The first, also from a research group in the United States, was conducted in 16 postmenopausal women with T2DM and in 19 age-matched non-diabetic controls. Significantly lower BMSi values were demonstrated in T2DM patients compared to controls, and there was a significant relationship between BMSi and the duration of T2DM (15). In addition, AGE accumulation in the skin as measured by skin autofluorescence, which has been previously shown to be a surrogate marker of AGE levels in bone, was significantly inversely correlated with BMSi in T2DM patients. This suggests that the accumulation of AGEs may also lead to impaired tissue-level properties of bone.

The second study was a population-based study conducted in 1053 elderly Swedish women aged 75–80 years with or without T2DM, of whom respectively 36% and 38% had sustained a fracture. Fifty-one women with T2DM and 438 without T2DM consented to have IMI measurements. BMSi was significantly lower in T2DM patients compared to controls (16).

Effect of glucocorticoid use on BMSi measurements

The only study that evaluated sequential changes in BMSi measurements was conducted in 52 Spanish subjects commencing treatment with glucocorticoids, a drug shown to rapidly lead to deterioration of bone tissue (17). Patients were allocated to treatment with different anti-osteoporosis agents based on Spanish national guidelines. 19 patients received only calcium/vitamin D supplementation (Ca/D), 14 risedronate (RSN), 14 denosumab (Dmab) and 5 teriparatide (TPTD). Measurements were performed at baseline, and at 7 and 20 weeks after start of treatment. After 7 weeks of treatment, there was a significant decrease in BMSi in the Ca/D3 group, no change in the RSN group, and an increase in BMSi in the Dmab and TPTD group, with no parallel change in BMD. However, the significant differences in demographic characteristics, glucocorticoid dose and different osteoporosis agents used, represent a significant limitation of this study. Patients were also pragmatically switched from the Ca/D group to an active treatment group if a 10% decrease in BMSi was observed in the first follow-up visit.

Geographical differences in BMSi

Duarte Sosa studied whether BMSi could differentiate between Norwegian and Spanish women, the former known to have increased risk for fracture not explained by BMD values (18). BMSi was shown to be significantly lower in 42 Norwegian women

compared to 46 Spanish women with the caveat that femoral neck BMD was significantly higher in Norwegian women. There was no relationship between BMSi and any other studied patient characteristic.

BMSi in patients with Human Immunodeficiency Virus

In a study that aimed at elucidating the mechanism of increased fracture risk associated with Human Immunodeficiency Virus (HIV), IMI was performed in 50 HIV patients [15 (30%) female] and 35 healthy controls [11 (31%) female] living in Spain (19). HIV patients had significantly lower BMSi values than controls. In these patients, BMSi was also significantly lower in women than in men [80 (IQR 77–83) vs. 85 (IQR 83–87); $p = 0.0004$]. In healthy control, there was no difference in BMSi values between sexes in healthy controls.

BMSi in kidney transplant recipients

In a further study from the same Spanish research group, 40 long-term kidney transplant recipients (58% female) and 94 controls (79% female) selected from a reference database of healthy controls subjects were evaluated using Trabecular Bone Score (TBS) and IMI (20). There was no relationship between BMD, TBS and BMSi values. Although BMSi values were significantly lower in renal transplant recipients compared to controls in the unadjusted analysis, this difference was no longer observed after adjusting for age, sex and BMI. The authors based their conclusion on their finding of similar tissue-level properties of bone in long-term kidney transplant recipients compared to control patients by conducting the final analyses using only adjusted BMSi values.

BMSi in chronic atrophic gastritis

Aasarod and colleagues evaluated Norwegian patients with chronic atrophic gastritis (CAG) compared to healthy controls to address the question whether CAG is associated with impaired material properties of bone (21). No difference in BMSi values was observed between CAG and control patients. The study was heavily underpowered, with BMSi values available in only 14 CAG patients and in 18 controls. The rationale for investigating bone material properties in this patient group was also not clear as the only mentioned potential contributory risk for altered material properties was the chronic gastric hypoacidity with this condition.

BMSi in acromegaly

Acromegaly is a rare endocrine disorder caused by overproduction of growth hormone associated with increased vertebral fracture risk in the presence of relatively normal BMD values. Our group demonstrated the presence of significantly lower mean

BMSi values in 48 acromegaly patients compared to 44 controls (22). There was no significant difference in BMSi between acromegaly patients with or without vertebral fractures.

BMSi in type 1 Gaucher disease

In the most recent study from the Spanish group, BMSi values were found to be significantly lower in 16 type 1 Gaucher disease (GD1) patients compared to 29 healthy volunteers (23). An inverse relationship was also observed between BMSi and chitotriosidase levels, a marker of GD activity, in keeping with the previously demonstrated relationship between BMSi and AGE accumulation in patients with T2DM.

BMSi and fracture risk

To date, four studies have evaluated the association between BMSi and prevalent fractures. Our group demonstrated that there was a strong negative relationship between BMSi and age, but not between BMSi and any other variable studied, including BMD, amongst 90 patients with low bone mass, 63 of whom had sustained a fragility fracture and 27 had not (24). BMSi was lower in patients who had sustained a fragility fracture than in patients who did not, in the presence of comparable BMD values (79.9 ± 0.6 vs. 82.4 ± 1.0 ; $p = 0.032$). However, after stratification for BMD, BMSi values were found to be lower in patients with osteopenia and fragility fractures than in patients with no fractures. There was no difference in BMSi values observed in patients with osteoporosis with or without fractures.

The study from Rudang and colleagues reported BMSi results of a population-based cohort of 75 to 80 year old Swedish postmenopausal women with ($n = 117$) or without fractures ($n = 63$) (25). They authors found a significant correlation between BMSi and areal BMD values at the lumbar spine, hip and distal radius. After adjustment for multiple covariates including, age, weight and height, use of bone-modifying agents and different operators, the only significant relationship which remained was between BMSi and BMD at the distal radius. BMSi was comparable in patients with or without fractures, also in the analysis that included only patients with osteopenia and osteoporosis (76.1 ± 6.9 vs. 75.1 ± 7.0 ; $p = 0.43$, respectively).

A further study from Norway specifically included women with stress fractures of the tibia but excluded patients with osteoporosis or previous low-energy fractures (26). Results from this study demonstrated significantly lower BMSi in patients with stress fractures than in controls without fractures (70.5 ± 8.7 vs. 77.1 ± 7.2 ; $p = 0.01$).

Finally, a study published by Duarte Sosa et al demonstrated that in 132 post-menopausal women, of whom 66 had sustained a fragility fracture at age ≥ 45 years or had osteoporosis at either the lumbar spine or femoral neck, and 66 had no fractures and normal BMD values, fracture patients had significantly lower BMSi values than no fracture controls (71.5 ± 7.3 vs. 76.4 ± 6.2 ; $p = 0.008$) (27). In the 24 patients with vertebral fractures, BMSi was inversely related with severity of vertebral fractures, also after adjustment for age and total hip BMD.

FUTURE PERSPECTIVES

Evidence has been accumulating on the value of IMI in the *in vivo* evaluation of tissue-level material properties of bone in humans. Current data highlight the ability of this technique in discriminating specific patient groups at risk for fractures from controls. Data on the value of IMI in the follow-up of patients with increased fracture risk remain inconclusive as available data are too scarce to provide adequate interpretation of results.

The technique is however not without limitations and so far available data suggest that some matters should be taken in consideration before recommending the use of IMI in the management of patients with increased bone fragility.

The Osteoprobe is a handheld device specifically designed for *in vivo* use in humans. The tool is relatively easy to use, but is also prone to variability in its results, in part because of the lack of a standard operating procedure. Data thus suggest that IMI is subject to inter-operator variability as shown in one study in which four different operators performed BMSi and reported variable results (25). This finding suggests that BMSi values may have to be operator-adjusted before allowing reliable comparison of data between different centers. Although it has been shown that IMI measurements can identify patients at increased risk for fractures, it remains unclear which specific mechanical properties of bone are captured by IMI. To our knowledge, only one study directly compared IMI with parameters obtained by the three-point bending technique and demonstrated no correlation between data obtained by the two techniques (28). Data from studies addressing the relationship between CMI and traditionally used mechanical tests provide contrasting results for instance regarding the relationship between CMI parameters and toughness (29–31). These data suggest that both IMI and CMI measure aspects of bone strength other than those measured by mechanical tests, and it is currently not known which aspects are precisely measured by RPI. The modest or non-existent correlation between CMI and IMI parameters suggests that the

two techniques measure different parameters and study results cannot therefore be extrapolated from one to the other (32).

One of the important limitations in the interpretation of IMI data is that study protocols vary in a number of key issues between centers. Study protocols differ in the number of measurements obtained per patient to calculate BMSi, and, even more significantly, different strategies are used to identify false measurements. This has led to the recent publication of a standard operating procedure for performing RPI including a recommended number of measurements and a protocol to be used to determine which measurements should be excluded from the calculation of BMSi (11). It is of utmost importance for the interpretation of BMSi results that all research groups using IMI adopt an identical study protocol so that results could be compared between centers. IMI is a simple, minimally invasive technique that is not time-consuming. Only two minor adverse events have been so far reported, one patient experienced a local allergic reaction to local anaesthetic (Lidocaine), and another patient developed a mild localized skin infection, which resolved after a short course of antibiotics.

A further complicating matter in the interpretation of BMSi results is the intriguing wide variability of BMSi values of control patients between studies. Some studies thus reported BMSi values of 80 or higher in control patients, whilst others reported lower BMSi values in the range of 70–78, corresponding to the range of BMSi values observed in patient groups in a number of studies identified to have increased fracture risk; Table 1. There are several potential causes for this discrepancy in findings. There might be a geographical difference in BMSi values as suggested by a study comparing BMSi values between Norwegian and Spanish women, which demonstrated significantly lower BMSi values in Norwegian women (18). Our group further consistently reported a highly significant relationship between BMSi and age, with a significant decrease in BMSi observed with increasing age (24, 33). The influence of age on BMSi may be particularly important in the interpretation of results from the study conducted by Rudang et al, where no relationship was found between BMSi and fracture in a population-based study in women aged 75–80 years (25). It can thus be hypothesized that BMSi values already significantly influenced by age may not be further significantly negatively influenced by the presence of prevalent fractures. These data suggest that reference values established for different ages and background are needed for the better interpretation of BMSi values, similar to the case with BMD and FRAX reference values.

A further matter of interest is whether IMI performed at the midshaft of the tibia may reflect tissue-level properties of bone at other skeletal sites than the tibia, thus reflect-

ing overall bone fragility rather than just cortical bone fragility. Interestingly, there are contrasting data on the relationship between BMSi and vertebral fractures. Whereas there was no relationship between BMSi and vertebral fractures in a population-based study of 211 older women, including 58 with vertebral fractures (25), a study of 132 postmenopausal women demonstrated significantly lower BMSi values in fracture patients (24 with vertebral fractures) compared to controls, also showing a significant relationship between BMSi and severity of vertebral fractures (27). Our group further demonstrated low BMSi values in patients with acromegaly, with or without vertebral fractures (22), and low BMSi values in fracture patients, independently of whether this was a vertebral fracture or a non-vertebral fracture (33), suggesting that BMSi may reflect overall bone fragility rather than just cortical bone fragility.

Based on currently published data, we can conclude that IMI captures elements of bone strength not captured by BMD in specific patient groups known to have increased fracture risk. IMI is not a measure of bone mass, and there is no clear relationship demonstrated between these two, suggesting that IMI should be considered as an additional tool in the assessment of bone health and not a replacement for BMD measurements. Large prospective clinical trials are required to determine whether BMSi values are of added value to currently used diagnostic tools such as DXA in the identification of patients with high fracture risk, and whether it is suitable for the prediction of treatment outcome in the management of osteoporosis. Results from these studies will determine whether IMI could be used in clinical practice or whether its use should be restricted for the time being to research settings until more evidence is gathered about its optimal use in the clinic.

Further experimental studies are also required to establish the relationship between IMI and traditional mechanical properties of bone.

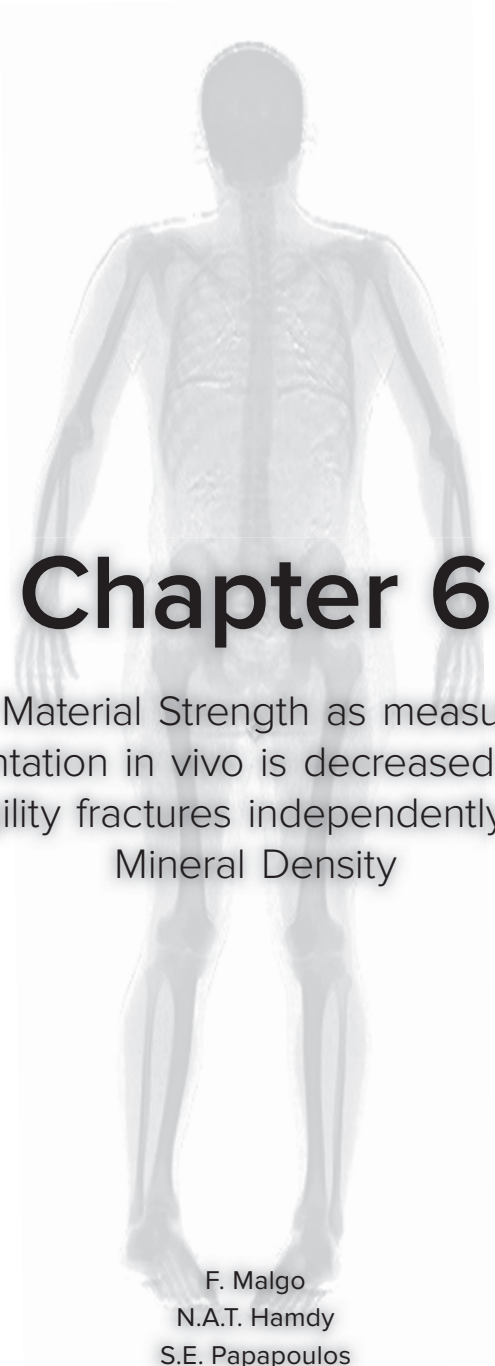
In conclusion, IMI is an exciting technique which enables researchers to evaluate tissue-level material properties of bone in a minimally invasive manner *in vivo*, adding to available tools such as DXA by capturing aspects of bone fragility not reflected by these currently used tools.

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Chapter 6

Bone Material Strength as measured by
microindentation in vivo is decreased in patients
with fragility fractures independently of Bone
Mineral Density

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ABSTRACT

Context:

Bone Mineral Density (BMD) does not fully capture fracture risk as the majority of fractures occur in patients with osteopenia, suggesting that altered bone material properties and changes in microarchitecture may contribute to fracture risk.

Objective:

To evaluate the relationship between Bone Material Strength (BMS), measured by microindentation in vivo, and fracture in patients with low bone mass.

Methods:

BMS was measured in 90 patients (mean age 61.0 years (range 40.4–85.5 years)) with low bone mass with or without a fragility fracture. Sixty-three patients had sustained one or more fragility fractures.

Results:

There was a significant negative correlation between age and BMS ($r = -0.539$, $p < 0.001$) and with the 10-year fracture probability with and without inclusion of femoral neck BMD as calculated by FRAX ($r = -0.383$, $p < 0.001$ and $r = -0.426$, $p < 0.001$, respectively). BMS values were lower in patients with a fragility fracture compared to non-fracture patients (79.9 ± 0.6 vs. 82.4 ± 1.0 , $p = 0.032$) despite similar BMD. BMS was comparable in patients with a fragility fracture whether they had osteopenia or osteoporosis (79.8 ± 0.8 vs. 78.7 ± 1.1 , $p = 0.456$). In patients with osteopenia, BMS was significantly lower in fracture patients than in non-fracture patients (80.3 ± 0.7 vs. 83.9 ± 1.2 ; $p = 0.015$).

Conclusion:

These data suggest that patients with fractures have altered material properties of bone which are not captured by BMD. Further studies are required to establish the value of BMS in the prediction of fracture risk, especially in patients with osteopenia.

INTRODUCTION

Osteoporotic fractures are common and their incidence rises with age, regardless of gender(1–3). All fractures represent a significant cause of morbidity and decreased quality of life, but fractures have also been shown to be associated with increased mortality(4, 5). There is mounting evidence that Bone Mineral Density (BMD) measurements using DXA only partially capture fracture risk, as a majority of fragility fractures have been shown to occur in patients with osteopenia(6, 7). This strongly suggests that determinants of bone strength other than bone mass may contribute to bone fragility in these patients. Such determinants would include changes in microarchitecture and of material properties of bone. Up until recently this hypothesis was, however, difficult to test in humans due to lack of appropriate techniques for evaluation of these determinants of bone strength.

Recent studies examining structural changes of bone in patients with fractures demonstrated a deterioration in bone microarchitecture(8, 9), as well as an association between increased cortical porosity and distal forearm fractures in patients with osteopenia(10). Reference point indentation is a new tool that permits *in vivo* measurements of bone material properties in humans. This technique has been extensively validated in animal models(11–13). Diez-Perez et al were the first to report data on microindentation *in vivo* in humans, showing a significantly higher indentation distance increase (IDI) in patients with osteoporotic fractures compared to non-fracture controls(14). Further development of the technique has led to the introduction of a handheld device to measure Bone Material Strength (BMS), a parameter derived from the ratio of the mean IDI between the calibration phantom and bone, as a quantifiable parameter of the ability of bone to resist microindentation(15). This device is inserted in the skin of the tibia until it reaches the bone surface and indents it. Using this technique, Farr et al recently reported that postmenopausal women with type 2 diabetes mellitus had lower BMS compared with age-matched non-diabetic controls, and suggested that this may contribute to the increased bone fragility observed in these patients(16).

The main objective of our study was to evaluate the relationship between BMS, as assessed by the microindentation *in vivo* technique, and fracture in patients with low bone mass.

PATIENTS AND METHODS

Study design

Cross-sectional study evaluating Bone Material Strength (BMS) using the microindentation in vivo technique in men and women attending the outpatient clinic of the Center for Bone Quality or the regional Fracture Liaison Service (FLS) of the Leiden University Medical Center between July 2013 and August 2014.

Patients

Patients were sequentially invited to take part in the study. Details of the recruitment process are shown in Supplemental Figure 1. Inclusion criteria included age between 40 and 85 years, low bone mass (osteopenia or osteoporosis as diagnosed by DXA) and willingness to be investigated using the microindentation in vivo technique. Exclusion criteria were a metabolic bone disorder other than osteoporosis, serum 25-OH vitamin D concentrations < 25 nmol/L, pathological fractures, severe liver or kidney impairment (CKD stage IV or V), current use of glucocorticoids, aromatase inhibitors, androgen deprivation therapy or chemotherapy and previous or current use of bone-acting agents (bisphosphonates, denosumab, selective estrogen receptor modulators (SERMs), strontium ranelate, recombinant PTH), immobilization, local infection of the tibia at the site of examination, bilateral hip replacement, participation in other research studies and inability to provide informed consent. Past use of glucocorticoids (longer than 3 months ago) was not an exclusion criterion as fracture risk has been shown to reverse quickly after discontinuation of treatment(17, 18). The Medical Ethics Committee of the LUMC approved the study and informed consent was obtained from all patients.

Methods

A full medical history including data on menopausal status, clinical risk factors for fractures for the calculation of the 10-year fracture probability (FRAX), a detailed fracture history with documentation of site and date of occurrence of the fracture, and information about use of medication were obtained from all patients. A fragility fracture was defined as any low energy fracture, excluding those of the hands, feet and skull. The FRAX probability for a major osteoporotic fracture and for a hip fracture was calculated using reference values for the Dutch population(19). Both fracture probabilities were computed with and without the inclusion of femoral neck BMD in the calculation. Fractures sustained < 12 months before the investigation were not included as a previous fracture in the calculation of the FRAX(20–22).

Serum Biochemistry

Blood samples were collected for the measurement of serum calcium, phosphate, albumin, creatinine, and liver enzymes using semi-automated techniques; serum 25-hydroxyvitamin D was measured using the 25-OH-vitamin D TOTAL assay (DiaSorin D.A./N.V., Brussels, Belgium) and plasma Intact PTH was measured by the immulite 2500 (Siemens Diagnostics, Breda, Holland).

Bone Mineral Density

Areal Bone Mineral Density (BMD) was measured at the lumbar spine (L1-L4) and at both femoral necks using Dual-energy X-ray absorptiometry (DXA) with the Hologic QDR 4500 (Hologic Inc., Waltham, MA, USA). Average values of the left and right femoral neck (FN) were used for analysis. T-scores were calculated using reference values of the National Health and Nutrition Examination Survey NHANES III and osteopenia and osteoporosis were diagnosed according to WHO criteria.

Radiographs of the spine

Lateral radiographs of the thoracic and lumbar spine were performed for the detection of vertebral deformities. All radiographs were independently evaluated by two of the authors using the semi-quantitative method of Genant(23).

Bone Material Strength

Bone Material Strength (BMS) was evaluated by microindentation *in vivo* using the Osteoprobe®, a Reference Point Indenter (kindly provided by Active Life Scientific Inc., Santa Barbara, CA, USA)(11, 14, 15). After local anaesthesia using a solution of 1% Lidocaine, the hand-held Osteoprobe® is inserted in the skin of the midshaft of the right tibia (mean distance between distal apex of the patella and medial malleolus) until it reaches the bone surface which is indented upon activation of the instrument. During measurements, the Osteoprobe® is maintained perpendicular to the surface of bone at the site of investigation. A minimum of five and up to twenty-five measurements were performed at the same site. During the procedure, the operator classified the sequential measurements as poorly, adequately or well performed, before checking the obtained data, to avoid reporter bias in the interpretation of results. After at least five adequate measurements in each subject, five additional measurements are performed on a polymethylmethacrylate (PMMA) plastic calibration phantom. BMS is calculated as 100 times the ratio of the mean indentation distance increase from impact into the PMMA calibration phantom divided by the indentation distance increase from impact into bone. The probe induces a microfracture as it indents the surface of the cortical bone of the tibia. The more easily this occurs, the deeper the probe indents the bone, and thus the lower the BMS (15). Coefficient of variation of the method was < 10% for different levels of BMS.

Statistical analysis

All analyses were performed using the SPSS software for Windows (Version 20.0; SPSS Inc., Chicago, IL, USA). All data are expressed as mean $\pm$ SD unless otherwise stated. Normality assumptions were checked by normality plots and by inspection of histograms of residuals from the various regression models. Between-group differences in baseline characteristics were assessed using a Student's *t*-test, a Chi-square test or a Mann-Whitney U test for non-normally distributed variables. Pearson's correlations were used to assess correlations between patients' parameters and BMS. Spearman's correlations were used to assess correlations between parameters that were not normally distributed and BMS values. Analysis of variance models with BMS as outcome variable, adjusted for covariates, were used to compare BMS values between groups. Binary logistic regression analysis was used to assess the separate contributions of BMS and femoral neck BMD (variables) to fracture (outcome). A probability level of random difference of 0.05 was considered significant.

RESULTS

Ninety of 125 eligible patients with low bone mass agreed to take part and were included in the study (Supplemental Figure 1). Forty-nine of them, all with fractures, were recruited from the FLS while 41, with or without fractures, were attending the outpatient clinic. Patients' characteristics and laboratory values are shown in Table 1. These were 53 women and 37 men, mean age 61.0 years (range 40.4 and 85.5 years), 61% of whom had osteopenia. Sixty-three patients (24 men) had sustained a low energy fracture (vertebral *n*=8; hip *n*=10; non-hip/non-vertebral *n*=45), in 43 of whom the fracture was recent. Microindentation was performed at a median time of 4.0 months after a fracture. Patients without history of a clinical fracture had also no radiological evidence for vertebral deformities on spinal radiographs.

Bone Material Strength (BMS) was significantly inversely related with age ($r = -0.539$, $p < 0.001$; Figure 1) and with the 10-year fracture probability with and without inclusion of femoral neck BMD in the calculation of FRAX ($r = -0.383$, $p < 0.001$ and $r = -0.426$, $p < 0.001$, respectively). BMS values were inversely and significantly related with age and with the 10-year fracture probability in both genders (age: women $r = -0.422$, $p = 0.001$, men $r = -0.570$, $p < 0.001$; FRAX: women $r = -0.286$, $p = 0.038$, men $r = -0.393$, $p = 0.016$). Because of the relationship between BMS and age all further reported values of BMS were adjusted for age. Unadjusted values are shown in Supplemental Table 1.

Table 1. Characteristics of 90 patients with low bone mass

	Fracture (n = 63)	No fracture (n = 27)	p-value
Age (years)	62.6 ± 9.6	57.1 ± 9.5	0.015
Male/female	24/39	13/14	0.374
BMI (kg/m ²)	24.3 ± 3.5	25.3 ± 4.7	0.725
Parental Hip Fracture (%)	9 (14%)	4 (15%)	0.948
Smoking (%)	14 (22%)	2 (7%)	0.092
Alcohol use > 3 IU/day (%)	14 (22%)	1 (4%)	0.031
Glucocorticoids (%)	4 (6%)	6 (22%)	0.028
FRAX probability			
Major fracture (%)	6.9 ± 1.0	4.0 ± 0.8	0.001
Hip fracture (%)	2.0 ± 0.8	1.0 ± 0.3	0.003
PTH (pmol/L)	3.8 ± 1.9	3.5 ± 1.5	0.570
Calcium (mmol/L)	2.41 ± 0.08	2.41 ± 0.10	0.826
25-OH D (nmol/L)	67.4 ± 28.6	79.6 ± 26.5	0.062
Creatinine (μmol/L)	73.5 ± 13.1	78.4 ± 15.0	0.163
LS BMD (g/cm ²)	0.87 ± 0.13	0.86 ± 0.12	0.402
T-score LS	-1.7 ± 1.2	-1.9 ± 1.1	0.431
FN BMD (g/cm ²)	0.67 ± 0.09	0.69 ± 0.08	0.303
T-score FN	-1.8 ± 0.7	-1.6 ± 0.6	0.329

Values are expressed as mean ± SD. FRAX is expressed as median ± SEM.

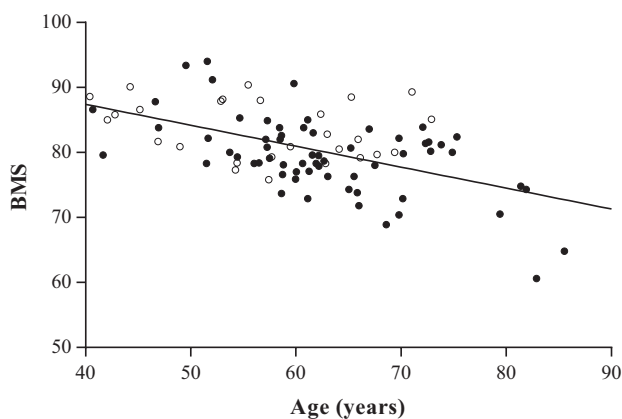
BMI Body Mass Index; PTH parathyroid hormone; LS lumbar spine; FN femoral neck;

BMD Bone Mineral Density.

Reference range PTH (0.7–8.0 pmol/L)

Reference range Calcium (2.15–2.55 mmol/L)

Reference range Creatinine (64–104 μmol/L for males, 49–90 μmol/L for females)

**Figure 1**

Relationship between age and Bone Material Strength (BMS) in 90 patients with osteoporosis or osteopenia. Closed circles represent patients with fragility fractures, open circles represent patients without fragility fractures. $r = -0.539$, $p < 0.001$.

BMS values did not differ between women and men (80.0 ± 0.7 vs. 81.6 ± 0.8 ; $p = 0.147$). There was no significant relationship between BMS and BMD (lumbar spine: $r = 0.129$, $p = 0.157$; femoral neck: $r = 0.134$, $p = 0.143$), BMI ($r = 0.075$, $p = 0.413$) or any of the biochemical parameters measured.

BMS in patients with low bone mass

BMS was comparable in patients with osteoporosis and those with osteopenia (79.9 ± 0.8 vs. 81.2 ± 0.7 , $p = 0.230$). Patients with osteoporosis were predominantly women, had significantly lower BMI and significantly lower lumbar spine and femoral neck BMD than patients with osteopenia.

Patients with osteoporosis and a history of fragility fracture ($n = 21$) were significantly older than those without a fragility fracture ($n = 14$) ($65.8 \text{ years} \pm 10.5$ vs. $53.7 \text{ years} \pm 10.0$; $p = 0.002$) and were more likely to be active smokers and/or to consume > 3 units of alcohol/day (29% vs. 0% ; $p = 0.028$ for either). All other measured parameters, including BMS (79.3 ± 1.3 vs. 80.7 ± 1.6 , $p = 0.540$) did not differ between the two groups; Figure 2.

In patients with osteopenia, there was no significant difference in clinical characteristics, serum biochemistry or BMD between fracture ($n = 42$) and non-fracture ($n = 13$) patients. However, BMS values were significantly lower in patients with fragility fractures compared to those without a fragility fracture (80.3 ± 0.7 vs. 83.9 ± 1.2 ; $p = 0.015$);

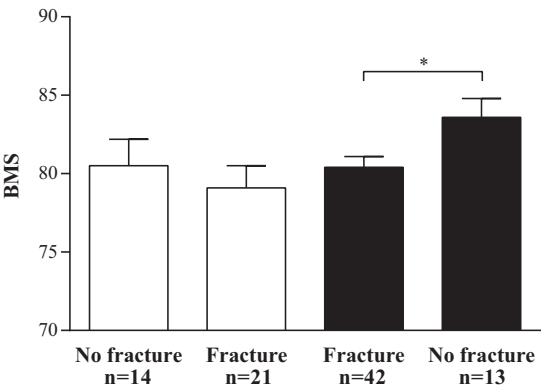


Figure 2

Bone Material Strength (BMS) in patients with osteoporosis (open bars) and osteopenia (closed bars), with and without fragility fractures. Mean $\pm$ SEM are shown.

* $p = 0.015$

Figure 2. This difference remained significant also after exclusion of patients with a hip fracture (80.4 ± 0.8 vs. 83.8 ± 1.2 ; $p = 0.027$).

BMS in patients with fragility fractures

BMS values were significantly lower in patients with fragility fractures compared to those who had never sustained a fracture (79.9 ± 0.6 vs. 82.4 ± 1.0 , $p = 0.032$), despite similar lumbar spine and femoral neck BMD values between the two groups; Figure 3. A lower BMS was associated with a higher odds for fractures [OR 1.15 (1.05–1.27), $p = 0.004$] whereas this was not the case for femoral neck BMD [OR 6.17 (0.02–2124.51), $p = 0.542$].

Among patients with a fragility fracture ($n = 63$), 42 had osteopenia and 21 osteoporosis; details shown in Table 2. BMS values were comparable in all patients with fragility fractures whether they had osteopenia or osteoporosis (79.8 ± 0.8 vs. 78.7 ± 1.1 , $p = 0.456$); Figure 2. There was no significant difference in BMS between patients who had sustained a leg fracture of the ipsilateral side of the measurement ($n = 8$) compared to those with a fracture of the contralateral side ($n = 10$) (77.6 ± 1.8 vs. 78.3 ± 1.6 , $p = 0.777$). Compared to patients with osteoporosis, patients with osteopenia comprised relatively more men and had higher BMI and BMD and lower 10-year fracture probability. All other clinical characteristics and biochemical measurements were similar between the two groups.

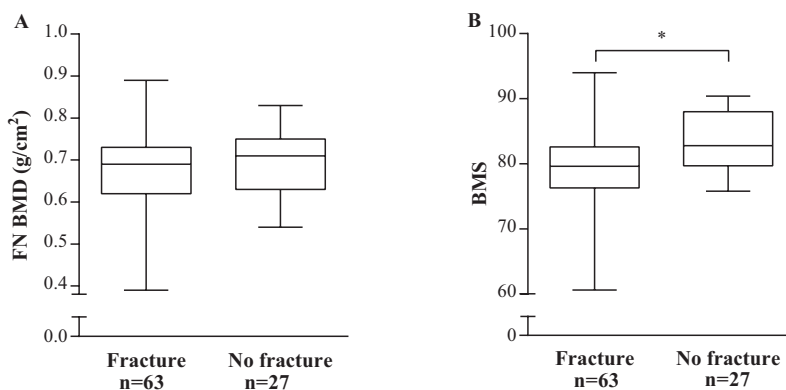


Figure 3

(A) Femoral neck Bone Mineral Density (FN BMD) and (B) Bone Material Strength (BMS) in patients with and without fragility fractures.

Data are shown in box-whisker plots and statistical differences are displayed for BMS. Boxes indicate median and interquartile range. Bars indicate minimum and maximum values.

* $p = 0.032$

Table 2. Characteristics of 63 patients with fragility fractures

	Osteopenia (n = 42)	Osteoporosis (n = 21)	p-value
Age (years)	61.0 ± 8.8	65.8 ± 10.5	0.058
Male/female	20/22	4/17	0.028
BMI (kg/m ²)	25.0 ± 3.2	22.8 ± 3.8	0.046
Parental Hip Fracture (%)	6 (14%)	3 (14%)	1.000
Smoking (%)	8 (19%)	6 (29%)	0.391
Alcohol use > 3 IU/day (%)	8 (19%)	6 (29%)	0.391
Glucocorticoids (%)	3 (7%)	1 (5%)	0.715
FRAX probability			
Major fracture (%)	6.5 ± 0.6	9.8 ± 2.6	0.004
Hip fracture (%)	1.4 ± 0.4	4.0 ± 2.2	0.001
PTH (pmol/L)	3.8 ± 1.8	3.9 ± 2.2	0.913
Calcium (mmol/L)	2.41 ± 0.08	2.41 ± 0.09	0.975
25-OH D (nmol/L)	69.2 ± 29.3	63.7 ± 27.5	0.474
Creatinine (μmol/L)	74.6 ± 12.1	71.5 ± 14.9	0.159
LS BMD (g/cm ²)	0.92 ± 0.12	0.77 ± 0.09	< 0.001
T-score LS	-1.3 ± 1.1	-2.6 ± 0.9	< 0.001
FN BMD (g/cm ²)	0.71 ± 0.07	0.60 ± 0.09	< 0.001
T-score FN	-1.5 ± 0.5	-2.3 ± 0.8	< 0.001

Values are expressed as mean ± SD. FRAX is expressed as median ± SEM.

BMI Body Mass Index; PTH parathyroid hormone; LS lumbar spine; FN femoral neck;

BMD Bone Mineral Density.

DISCUSSION

We show here that patients who had sustained a fragility fracture demonstrate a significantly lower Bone Material Strength, as measured by the microindentation in vivo technique, compared to patients who did not fracture. More importantly, our data also demonstrate that there was no difference in Bone Material Strength in patients with fragility fractures whether they had osteopenia or osteoporosis. Our findings thus suggest that bone material properties are altered in patients with a fragility fracture and that the microindentation in vivo-derived BMS measurement captures elements of bone fragility independently of BMD. Analysis of data on bone turnover markers, which may also be associated with an increase in fracture risk independently of BMD(24), were not undertaken in this study because reliable interpretation of the data was precluded by the large number of patients with a recent fracture and the influence of this on serum levels of these markers.

Microindentation *in vivo* is a new technique, designed to measure the resistance of bone to fracture by separating mineralized collagen microfibers and thus, locally inducing microcracks. In the first human studies, the material properties of bone were quantified by Total Indentation Distance, Indentation Distance Increase (IDI), and creep Indentation Distance(14, 25). Of these parameters, IDI differentiated best between bone which was easily susceptible to fracture, and bone that did not easily fracture and the parameter was found to correlate best with toughness of bone(11, 13). This has led to the development of the derived parameter of Bone Material Strength (BMS), which is calculated by the ratio of the IDI of the calibration material PMMA to the IDI of bone(15).

In our study, we found a strong relationship between BMS and age, which may play an important role in the increased fracture risk observed in elderly patients, in whom deteriorated bone microarchitecture has also been demonstrated(8). Several *ex vivo* studies have shown an inverse relationship between age and toughness of bone(26, 27). Bone toughness is best predicted by the Indentation Distance Increase and thus by BMS(13). As bone strength is inversely correlated with the density of microcracks in bone tissue, the observed alteration in bone material properties in the elderly might well be explained by the previously demonstrated age-related accumulation of microcracks(26, 27).

Having established that BMS reflected bone fragility independently of BMD, we went on to test the association between BMS and the clinical risk factors used in the FRAX algorithm without inclusion of BMD measurements in the calculation. We found a significant relationship between BMS and the 10-year fracture probability calculated by FRAX without BMD, probably reflecting the lack of correlation between BMS and BMD values. These observations suggest that microindentation *in vivo* is able to capture an element of the contribution of clinical risk factors used in the FRAX algorithm to altered material properties of bone, and thus to increased fracture risk.

Our data complement and extend those of Diez-Perez et al, who, using the microindentation technique, showed that bone material properties, as measured by IDI, were poorer in 27 postmenopausal women who had sustained mainly a hip fracture ($n = 25$) or vertebral fracture ($n = 2$), compared to age-matched controls who had not sustained a fracture, with the caveat that women in the control group had a higher BMD than the fracture patients(14).

Postmenopausal women have altered bone microarchitecture, and more recent data showed that osteopenic women who sustained a fracture had worse bone microar-

chitecture than non-fracture controls(8, 9). Increased cortical porosity has also been suggested to contribute to the risk of distal forearm fractures in postmenopausal women with osteopenia(10). Our data provide evidence that bone material properties are altered in patients with osteopenia who have sustained a fracture, the majority of whom are currently not being offered treatment with bone-modifying agents.

Our study has strengths as well as limitations. We sequentially investigated patients of both genders with a wide age range and low bone mass reflecting everyday clinical practice. The frequency of osteopenia and osteoporosis within the group of patients with a fragility fracture is further consistent with previous reports in fracture patients(6, 7) and all measurements were performed by two dedicated operators. Furthermore, age and gender of patients enrolled in the study did not differ from those who were not investigated. A limitation of our study is that, whereas the main source of the fragility fracture patients included in the study was our regional Fracture Liaison Service, the non- fragility fracture controls were recruited from patients routinely attending our outpatient clinic, in whom bone mineral density measurements were requested at the discretion of the treating physician, possibly creating a selection bias.

In conclusion we demonstrate in this study that Bone Material Strength, as measured by the microindentation in vivo technique, captures elements of bone fragility such as the effect of aging and that of the cumulative effect of clinical risk factors as calculated by the FRAX algorithm, independently of BMD. Furthermore, we demonstrate that Bone Material Strength is comparable in patients with a fragility fracture, whether they have osteoporosis or osteopenia. These data suggest an aspect of altered bone quality contributing to bone fragility which is not captured by BMD. Further studies are required to establish the value of BMS as a predictor of fracture risk, especially in patients with osteopenia.

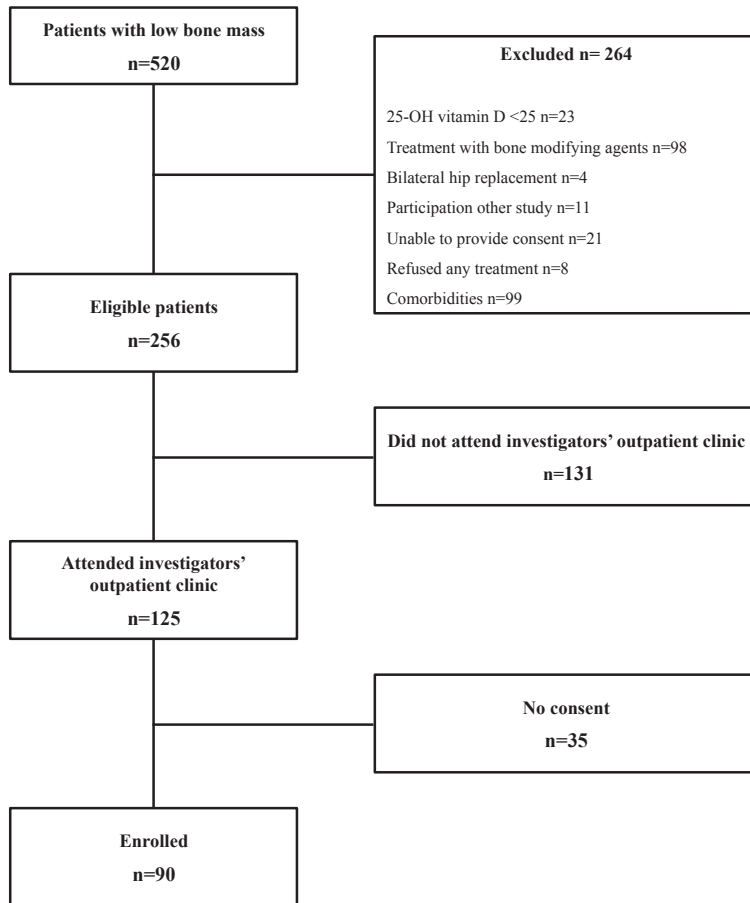
ACKNOWLEDGEMENTS

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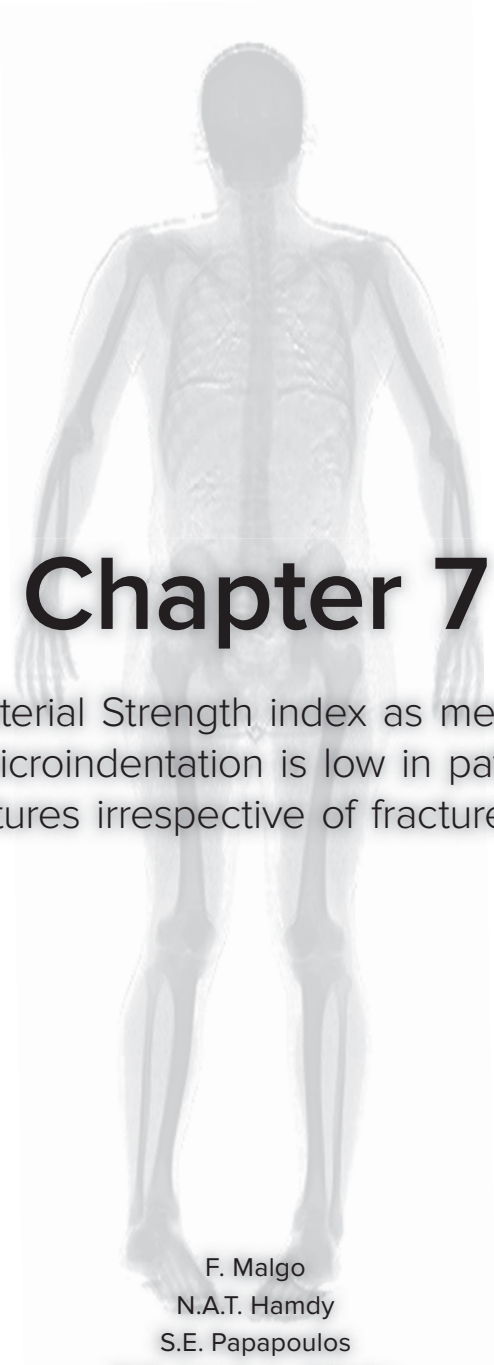
Supplemental Figure 1

Supplemental Table 1. Unadjusted and adjusted BMS values

	Osteoporosis Fx+	Osteoporosis Fx-	Osteopenia Fx+	Osteopenia Fx-	Fracture	No fracture
Unadjusted BMS	77.6 ± 1.4	83.2 ± 1.7	80.3 ± 0.7	83.9 ± 1.3	79.4 ± 0.7	83.6 ± 1.1
Adjusted BMS	79.3 ± 1.3	80.7 ± 1.6	80.3 ± 0.7	83.9 ± 1.2	79.9 ± 0.6	82.4 ± 1.0

Values are expressed as mean ± SEM.

Fx+ fracture patients; Fx- non-fracture patients; BMS Bone Material Strength.



Chapter 7

Bone Material Strength index as measured by impact microindentation is low in patients with fractures irrespective of fracture site

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ABSTRACT

Summary:

We evaluated the relationship between BMSi and fragility fractures, including vertebral fractures. Our data showed that BMSi is low in all fracture patients with low bone mass, independently of whether patients sustained a vertebral or a non-vertebral fracture.

Introduction:

Impact microindentation (IMI) is a new technique for the measurement of tissue-level properties of cortical bone in vivo. Previous studies showed an association between Bone Material Strength index (BMSi) and non-vertebral fractures but an association with vertebral fractures is still being debated.

Objective:

To evaluate the relationship between BMSi and different types of fragility fractures, including vertebral fractures.

Methods:

In this cross-sectional study, we measured BMSi in patients of both sexes with different types of fragility fractures and low bone mass with the IMI method using the Osteo-probe[®]. Vertebral fractures were diagnosed and graded on lateral spine radiographs.

Results:

132 patients were included in the study, of whom 101 patients (65 women) had sustained a low energy fracture and 31 (mean age 57.7 ± 9.9 years) had no history or radiological evidence for a fracture. Of the fracture patients, 53 (mean age 62.8 ± 8.3 years) had only non-vertebral fractures (VF-/Fx+), 34 (mean age 62.8 ± 9.9 years) had vertebral and non-vertebral fractures (VF+/Fx+) and 14 (mean age 64.7 ± 9.3 years) had only vertebral fractures (VF+/Fx-). BMSi values, adjusted for age and BMD, were similar for all 3 groups of fracture patients (78.9 ± 0.7 , 78.3 ± 0.9 and 78.4 ± 1.4 , respectively; $p = 0.866$). BMSi values were not associated with number or severity of vertebral fractures.

Conclusion:

Our data demonstrate that BMSi is low in fracture patients with low bone mass, irrespective of whether they sustained a vertebral fracture or a non-vertebral fracture.

INTRODUCTION

Reference Point Indentation (RPI) is a technique to measure cortical bone indentation properties *in vivo* and to potentially assess bone mechanical properties. Currently available RPI devices use two different methods to assess cortical bone resistance to indentation namely cyclic microindentation (CMI, Biodent) and impact microindentation with an easier to use hand-held indenter (IMI, OsteoProbe), the latter being specifically designed for *in vivo* use in humans and large animals [1–3]. The principles, similarities and differences as well as results of clinical application of the two devices were recently reviewed [4–6]. In a direct comparison of the two methods in cadaveric human tibiae, Karim and colleagues reported that results of measurements are weakly related indicating that each device reflects different aspects of cortical bone indentation properties [7]. Although the exact properties of bone measured *in vivo* by microindentation need to be determined, clinical studies with both devices demonstrated that the method could differentiate patient groups with increased fracture risk from control groups independently of Bone Mineral Density (BMD) values suggesting that the method measures aspects of bone fragility not captured by BMD.

Initial application of CMI in humans showed that postmenopausal women with osteoporotic fractures (hip or vertebra) or those with atypical femoral fractures had greater indentation distances compared with controls, that were poorly related with hip BMD [2, 8]. Clinical studies with IMI demonstrated that the measured parameter Bone Material Strength index (BMSi) was significantly lower in postmenopausal women with type 2 diabetes mellitus compared with healthy controls in the presence of similar BMD values [9], and patients treated with glucocorticoids showed significant deterioration of BMSi within 7 weeks of starting treatment, before any changes in BMD [10]. Our group also showed that despite similar BMD values, BMSi values were lower in patients with fragility fractures compared with those without fractures [11].

The impact microindentation technique has recently been used to examine the association between BMSi and vertebral fractures, but results are conflicting. In a population-based cohort of 211 older women with or without fractures, including 58 women with vertebral fractures, there was no association between BMSi values and prevalent vertebral fractures [12]. In contrast, a preliminary communication of a study in 101 postmenopausal women (30 with vertebral fractures) reported lower BMSi values in patients with fractures than in healthy controls and a significant inverse relationship between BMSi and severity of vertebral fractures [13].

The question raised by these studies is whether BMSi as measured by IMI provides information on just cortical bone fragility or on overall bone fragility. To address this question, we expanded our previously reported cohort to specifically examine the association between BMSi and different types of fragility fractures, in particular those of the vertebrae.

PATIENTS AND METHODS

This was a cross-sectional study that evaluated Bone Material Strength index (BMSi) in men and women attending the outpatient clinic of the Center for Bone Quality of the Leiden University Medical Center. The patients described in the current study include those of our previous report of the comparison of BMSi between patients with fragility fractures and those without fractures [11]. The study design, inclusion and exclusion criteria and methodology used were previously described [11] and are summarized below. The Medical Ethics Committee of the LUMC approved the study and informed consent was obtained from all patients.

Sequentially invited patients aged between 40 and 85 years with low bone mass (osteopenia or osteoporosis), and no secondary osteoporosis or other metabolic bone disease were invited to participate in the study. Other exclusion criteria included serum 25-OH vitamin D concentrations ≤ 25 nmol/l, current use of glucocorticoids, aromatase inhibitors, androgen deprivation therapy or chemotherapy or ever use of bone-acting agents such as bisphosphonates or denosumab. Fragility fractures were defined as any low-energy fracture, excluding those of hands, feet and skull. FRAX 10-year probabilities for a major osteoporotic fracture and a hip fracture, with and without femoral neck BMD, were calculated using the FRAX algorithm [14]. Blood was collected for standard laboratory investigations including calcium, creatinine, parathyroid hormone and 25-OH vitamin D. BMD was measured by DXA (Hologic QDR 4500) and conventional lateral radiographs of the thoracic and lumbar spine were obtained. Vertebral fractures were identified and graded according to the semi-quantitative method of Genant by two of the authors (F.M. and N.A.-D.) [15]. Kappa was 0.85.

BMSi was measured in all patients by impact microindentation (IMI) [3, 5] on the midshaft of the right tibia using a hand-held microindenter (OsteoProbe® RUO, Active Life Scientific, CA, USA) by a single operator [11]. The patient is 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 is defined as the mean distance between the distal apex of the patella and medial malleolus. Following disinfection of the area and local anaesthesia of the skin and periosteum with Lidocaine 1%, the test probe is gently inserted in the skin until the bone surface is reached. The operator ensures that the test probe is placed perpendicularly to the bone surface and classifies the measurements as “well performed”, “adequate” or “poorly performed” after the indentation and before checking the computer display of the result. “Poorly performed” measurements are usually due either to slipping of the test probe or to moving of the subject’s leg and are discarded. After five adequate measurements, as

recommended [5], five additional measurements are performed on a polymethylmethacrylate (PMMA) calibration phantom. BMSi is calculated as 100 times the harmonic mean of the indentation distance increase from impact into the PMMA material divided by the average indentation distance increase from impact into bone. As the probe indents the surface of the cortical bone of the tibia, it induces a microfracture. The more easily this occurs, the deeper the probe indents the bone, and thus the lower the BMSi is [3].

In 10 subjects the measurement was repeated in the same leg at sites 2 cm apart; mean (SD) of the two measurements was 75.9 ± 5.7 and 76.6 ± 4.9 , respectively. The intra-observer coefficient of variation (CV) was 2.2%.

Statistical analysis

All data are expressed as mean $\pm$ SD unless otherwise stated. Between-group differences in baseline characteristics were assessed by Student's t-test, ANOVA and Chi-square test or a Mann-Whitney U test or Kruskal-Wallis test for non-normally distributed variables. Post-hoc analyses using Bonferroni were performed to adjust for multiple comparisons if required and non-parametric post-hoc analyses using Kruskal-Wallis were performed to compare BMSi values between the three fracture groups and non-fracture controls. Pearson's or Spearman's correlations were used to assess correlations between patients' parameters and BMSi. ANOVA with BMSi as outcome variable, adjusted for age was used to compare BMSi values between patients with fractures and those without fractures. The relationship between BMSi and the severity of vertebral fractures was assessed by a linear multiple regression model adjusted for age and femoral neck BMD. A probability level of random difference of 0.05 was considered significant. The SPSS software for Windows (Version 23; SPSS Inc., Chicago, IL, USA) was used for statistical analysis of results.

RESULTS

One hundred and thirty-two patients (64% women), 101 of whom had sustained at least one fragility fracture, consented to take part in the study and underwent impact micro-indentation, a median of 3.3 months after their most recent fracture; Table 1. Mean age of the whole group was 61.8 ± 9.4 years, 76 patients (58%) had osteopenia and 56 (42%) osteoporosis. Consistent with our previous findings, in this larger cohort, we found that BMSi was inversely and significantly related with age ($r = -0.485$, $p < 0.001$) and with FRAX 10-year probability calculated with and without femoral neck BMD (Spearman's $r = -0.313$, $p < 0.001$ and Spearman's $r = -0.356$, $p < 0.001$, respectively). There was no correlation between BMSi with any other patient characteristic, laboratory or BMD

Table 1. Demographic characteristics of 132 patients with low bone mass

	Fracture (n = 101)	No fracture (n = 31)	p-value
Age (years)	63.1 ± 8.9	57.5 ± 9.9	0.004
Male/female	36/65	11/20	0.987
BMI (kg/m ²)	24.6 ± 3.5	25.1 ± 4.7	0.475
PTH (pmol/L)	3.6 ± 1.8	3.4 ± 1.4	0.618
Calcium (mmol/L)	2.41 ± 0.09	2.40 ± 0.10	0.784
25-OH D (nmol/L)	66.2 ± 27.1	78.1 ± 30.0	0.080
Creatinine (μmol/L)	73.4 ± 12.3	76.7 ± 15.2	0.228
LS BMD (g/cm ²)	0.87 ± 0.12	0.83 ± 0.08	0.090
FN BMD (g/cm ²)	0.67 ± 0.08	0.68 ± 0.07	0.672

BMI Body Mass index; PTH parathyroid hormone; LS lumbar spine; FN femoral neck; BMD bone mineral density.

value. There was no difference in BMSi values between women and men (79.7 ± 0.6 vs. 80.0 ± 0.8 ; $p = 0.789$). In the whole cohort of subjects with low bone mass, patients with fractures had significantly lower age-adjusted BMSi values than those without fractures (79.0 ± 0.5 vs. 82.5 ± 0.9 ; $p = 0.001$).

53 patients had only non-vertebral fractures (VF-/Fx+), 34 had non-vertebral and vertebral fractures (VF+/Fx+), and 14 had only vertebral fractures (VF+/Fx-); Table 2. Clinical characteristics and biochemical findings were similar among the three groups

Table 2. Demographic characteristics of 101 patients with low bone mass and fragility fractures

	VF+/Fx- (n = 14)	VF+/Fx+ (n = 34)	VF-/Fx+ (n = 53)	p-value
Age (years)	64.7 ± 9.3	62.8 ± 9.9	62.8 ± 8.3	0.761
Male/female	8/6	14/20	14/39	0.073
BMI (kg/m ²)	25.4 ± 3.1	24.7 ± 3.6	24.3 ± 3.6	0.574
PTH (pmol/L)	4.3 ± 2.0	4.0 ± 1.8	3.2 ± 1.6	0.021
Calcium (mmol/L)	2.39 ± 0.10	2.41 ± 0.11	2.42 ± 0.08	0.657
25-OH D (nmol/L)	64.8 ± 24.6	67.1 ± 28.0	66.0 ± 27.6	0.932
Creatinine (μmol/L)	79.4 ± 10.3	74.3 ± 11.7	71.2 ± 12.8	0.078
LS BMD (g/cm ²)	0.86 ± 0.11	0.88 ± 0.16	0.87 ± 0.10	0.833
FN BMD (g/cm ²)	0.70 ± 0.09	0.69 ± 0.09	0.65 ± 0.07	0.010 ^a

VF+/Fx- only vertebral fractures; VF+/Fx+ vertebral fractures and non-vertebral fractures; VF-/Fx+ only non-vertebral fractures; BMI Body Mass index; PTH parathyroid hormone; LS lumbar spine; FN femoral neck; BMD bone mineral density.

^apost-hoc notations FN BMD: VF+/Fx+ vs. VF-/Fx+; $p = 0.05$

with the exception of PTH values and femoral neck BMD which were lower in the (VF-/Fx+) group.

To evaluate possible differences in fracture risk due to different fracture history we analysed the number and type of prevalent non-vertebral fractures in the two groups. Twenty-four (71%) patients in the (VF+/Fx+) group and 31 (59%) patients in the (VF-/Fx+) group had only one prevalent non-vertebral fracture; ($p = 0.254$). The number of patients with hip fractures did not differ between groups (6 vs. 7 respectively). Similarly, there was no difference between the two groups in the total number of prevalent non-vertebral fractures, [48/34 (1.4 per patient) vs. 76/53 (1.4 per patient)] or major non-vertebral fractures [34/34 (1.0 per patient) vs. 45/53 (0.8 per patient)].

BMSi and vertebral fractures

BMSi values were similar in the three fracture groups, and significantly lower than those of the non-fracture control group; Figure 1. Results remained unchanged after exclusion of patients with mild (grade 1) vertebral fractures from the analysis.

We further examined the relationship between BMSi and the number and severity of vertebral fractures as classified using Genant's grading of vertebral fractures. There were 28 patients with 1 vertebral fracture, 9 with two vertebral fractures, and 11 had 3 or more vertebral fractures. There was no difference in BMSi in patients with 1, 2, or ≥ 3 vertebral fractures (78.6 ± 0.9 , 76.8 ± 1.6 , 79.0 ± 1.4 ; $p = 0.555$).

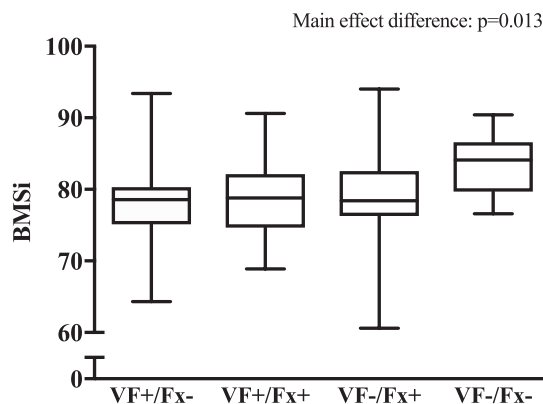


Figure 1

Bone Material Strength index (BMSi) in patients with only vertebral fractures (VF+/Fx-), vertebral fractures and non-vertebral fractures (VF+/Fx+), only non-vertebral fractures (VF-/Fx+) and in control patients (VF-/Fx-). Data are shown as box-whisker plots. Boxes indicate median and interquartile range. Bars indicate minimum and maximum values.

Main effect difference: $F = 3.74$; $p = 0.013$. Post-hoc notations: VF+/Fx- vs. VF-/Fx-; $p = 0.004$, VF+/Fx+ vs. VF-/Fx-; $p = 0.001$, VF-/Fx+ vs. VF-/Fx-; $p < 0.001$

Eighteen patients had a grade 1 vertebral fracture as their highest graded fracture, 20 had grade 2 and 10 had grade 3. There was no relationship between BMSi values and the severity of vertebral fractures, ($\beta = 0.826$; $p = 0.375$).

DISCUSSION

The present study confirms our previous results demonstrating that BMSi is lower in patients who had sustained one or more fragility fractures compared to patients who never sustained a fracture, independently of BMD measurements. It also demonstrates that vertebral fractures are associated with low BMSi values, which are not further negatively affected by the additional presence of non-vertebral fractures, including hip fractures. Taken together, these findings suggest that BMSi as measured by IMI on the tibia is associated with increased bone fragility at all relevant skeletal sites.

Our results are in keeping with those of Duarte Sosa et al who reported in a preliminary communication that postmenopausal women with fractures, including those of the spine, had significantly lower BMSi values than controls [13]. Our data are at odds, however, with those of Rudang et al who reported no difference in BMSi values between women with and without fractures, and no association between BMSi and vertebral fractures [12]. Various reasons may be responsible for the discrepancy in results between the latter study and ours, all related to major differences in design and methodology. Firstly, Rudang and colleagues studied elderly women, aged between 75 and 80 years, who were recruited from a population cohort while we included a clinically-based cohort of men and women aged between 40 and 85 years in whom we demonstrated a significant relationship between age and BMSi values. Secondly, broad inclusion criteria were used in this study compared to the strict selection criteria for inclusion in our study, an important prerequisite for studies using new methodologies. Thirdly, in our study the presence of fractures was radiologically confirmed in all patients compared to information about non-vertebral fractures obtained by questionnaire and not confirmed by review of medical records or radiographs. Finally, subjects with traumatic fractures who were specifically excluded in our study were included although it is not yet clear whether such fractures are associated with altered bone composition. Moreover, in our study BMSi measurements were performed by a single operator compared to 4 different operators in the study of Rudang and colleagues leading to the need to adjust their data because of the significant differences in the results obtained.

It should be also noted that differences in BMSi values were observed in a study of 42 Norwegian and 46 Spanish women suggesting possible geographical variations in

BMSi [16]. These differences in BMSi between countries suggest that, similar to FRAX, normative values for BMSi should perhaps be developed for different countries to minimize the implications of these differences in the interpretation of BMSi results.

Our study has strengths as well as limitations. Of the strengths of the study is the consecutive inclusion of men and women aged between 40 and 85 years with low bone mass attending our outpatient clinic. Although this is a main strength in our group of patients with fractures, it may be also perceived as a limitation of our patient control group because BMD measurements were performed at the discretion of the patients' physician. A further strength of our study is that patients who were currently using or had used bone-modifying agents were excluded, although this might also have resulted in the exclusion of patient known to have a high fracture risk. Of the limitations of our study are its cross-sectional design and the limited sample size after stratification for fracture type.

In conclusion, we show that Bone Material Strength index measured by IMI is low in patients with fragility fractures regardless of whether this is a vertebral or a non-vertebral fracture. These data suggest that BMSi may reflect general bone fragility although this remains to be established in prospective studies.

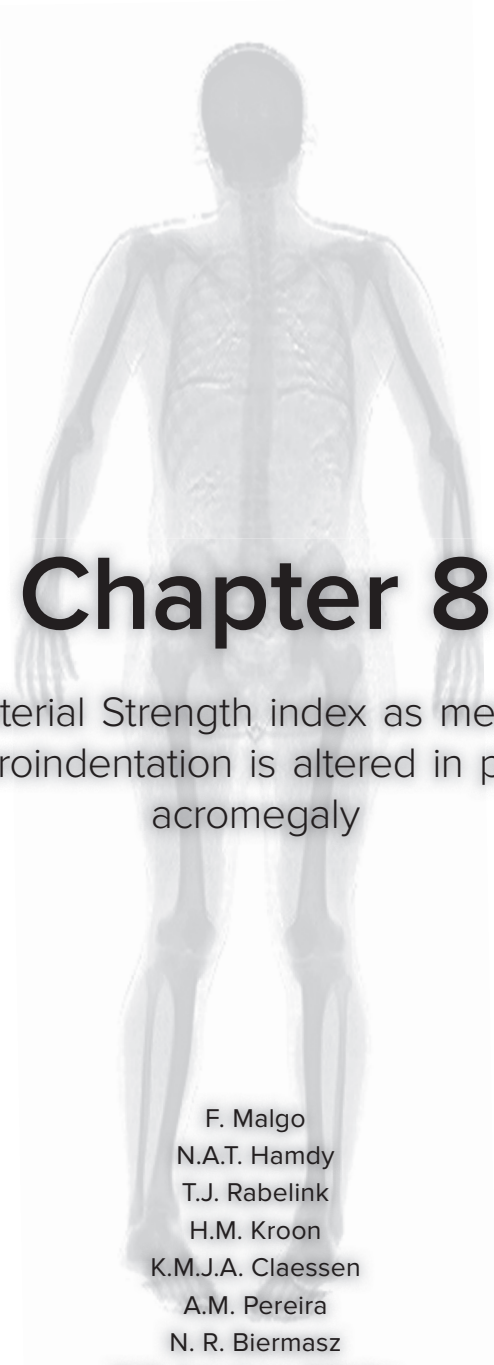
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Chapter 8

Bone Material Strength index as measured by
impact microindentation is altered in patients with
acromegaly

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ABSTRACT

Objective:

Acromegaly is a rare disease caused by excess growth hormone (GH) production by a pituitary adenoma. The skeletal complications of GH and IGF-1 excess include increased bone turnover, increased cortical bone mass and deteriorated microarchitecture of trabecular bone, associated with a high risk of vertebral fractures in the presence of relatively normal Bone Mineral Density (BMD). We aimed to evaluate tissue-level properties of bone using impact microindentation (IMI) in well-controlled patients with acromegaly aged ≥ 18 years compared to 44 controls from the outpatient clinic of the Center for Bone Quality.

Design and methods:

In this cross-sectional study, Bone Material Strength index (BMSi) was measured in 48 acromegaly patients and 44 controls with impact microindentation using the Osteo-probe®.

Results:

Mean age of acromegaly patients (54% male) was 60.2 years (range 37.9–76.5), and 60.5 years (range 39.8–78.6) in controls (50% male). Patients with acromegaly and control patients had comparable BMI ($28.2\text{kg/m}^2 \pm 4.7$ vs. $26.6\text{kg/m}^2 \pm 4.3$, $p = 0.087$), and comparable BMD at the lumbar spine ($1.04\text{g/cm}^2 \pm 0.21$ vs. $1.03\text{g/cm}^2 \pm 0.13$, $p = 0.850$) and at the femoral neck ($0.84\text{g/cm}^2 \pm 0.16$ vs. $0.80\text{g/cm}^2 \pm 0.09$, $p = 0.246$). BMSi was significantly lower in acromegaly patients than in controls (79.4 ± 0.7 vs. 83.2 ± 0.7 ; $p < 0.001$).

Conclusion:

Our data indicate that tissue-level properties of cortical bone are significantly altered in patients with controlled acromegaly after reversal of long-term exposure to pathologically high GH and IGF-1 levels. Our findings also suggest that methods other than DXA should be considered to evaluate bone fragility in patients with acromegaly.

INTRODUCTION

Acromegaly is a rare endocrine disease characterized by excess circulating growth hormone (GH) and Insulin-like Growth Factor-1 (IGF-1) levels, usually caused by an adenoma of the anterior pituitary gland. GH excess results in morbidity of multiple organ systems, including generalised tissue hypertrophy, cardio-metabolic disorders (diabetes mellitus, hypertension and cardiomyopathy) and arthropathy. Acromegaly has been shown to be associated with an increased risk of vertebral fractures in the presence of a relatively normal Bone Mineral Density (BMD), and it has also been shown that these fractures may progress despite adequate control of disease activity(1–5). Patients with active acromegaly demonstrate high bone turnover associated with increased cortical BMD in the presence of stable trabecular bone mass. Iliac crest biopsies indeed show an increase in bone remodelling compared to healthy controls(6). Trabecular connections are lost, due to the high bone turnover, resulting in altered bone microarchitecture, which persists also after successful induction of remission and restoration of bone remodelling rates(7, 8). In a study with HRpQCT, structural measurements confirmed this altered bone microarchitecture in patients with active as well as controlled acromegaly. This included deteriorated trabecular microarchitectural parameters in the distal radius and distal tibia, and increased cortical volumetric BMD in the distal tibia in patients with active disease(9). Areal BMD is often reported to be normal in these patients, although some studies do suggest an association with low bone mass(2, 10). Overall, current methods do not provide an adequate explanation for the increased fracture risk in these patients. However, in a recently published study evaluating the association between bone microstructure, as measured by high-resolution cone beam computed tomography, and vertebral fractures, the authors found deteriorated microstructure in both the trabecular and the cortical bone compartment of acromegaly patients with vertebral fractures compared to those without vertebral fractures(11).

There are no published data available on the assessment of material properties of cortical bone in patients with acromegaly, however, there are published data on material properties of trabecular bone in patients with acromegaly(12). The technique of impact microindentation (IMI) was introduced as a Reference Point Indentation method to acquire Bone Material Strength index measurements *in vivo*, and is used as a surrogate to assess tissue-level properties of bone. Previous studies have shown deteriorated material properties in postmenopausal women with osteoporotic fractures, atypical femoral fractures, with type 2 diabetes mellitus, and in patients with fragility fractures. (13–16). Although IMI reflects cortical bone properties rather than trabecular bone properties there is evidence that the elevated GH levels seen in acromegaly affect both compartments(6, 11). As current diagnostic tools do not adequately identify

acromegaly patients, which are at high risk for vertebral fractures, new methods are needed.

In this study, we aimed to evaluate previously reported findings of poor bone material properties in patients with acromegaly by evaluating whether BMSi is different between patients with acromegaly in remission and controls without acromegaly. In addition, we also aimed to evaluate whether BMSi was different in patients with acromegaly, with or without vertebral fractures.

PATIENTS AND METHODS

Study design

We performed a cross-sectional study to compare Bone Material Strength index (BMSi) between patients with well-controlled acromegaly and non-acromegaly control patients with osteopenia or normal Bone Mineral Density (BMD), with or without fractures. Patients were studied at the outpatient clinics of the Leiden University Medical Center. The Medical Ethics Committee of the Leiden University Medical Center reviewed and approved the study, and all patients gave written informed consent to participate in the study.

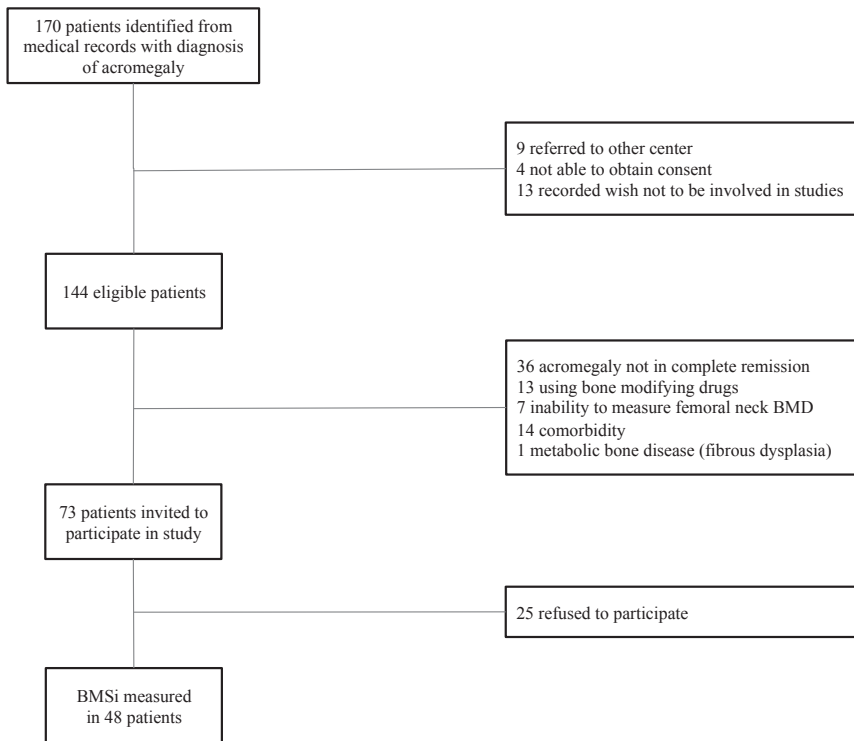
Patients with acromegaly

All patients aged 18 years or older with well-controlled acromegaly attending the outpatient clinic of the Department of Endocrinology of the Leiden University Medical Center were identified from hospital records. 170 patients with acromegaly, 73 of whom fulfilled the inclusion criteria and were invited to participate in the study. Forty-eight patients responded positively and were included in the study; Fig. 1. Of these patients, 26 (54%) had been evaluated in previously reported studies from our center(2, 4).

In this study, well-controlled acromegaly was defined as serum IGF-1 levels within the normal age range after surgery, radiotherapy, medical treatment or a combination thereof, and when required additional confirmation of remission from glucose-suppression tests. Oral glucose tolerance tests were performed yearly to assess disease activity, except in patients receiving medical treatment(17).

Controls

Controls were recruited from the outpatient clinic of the Center for Bone Quality or from the regional Fracture Liaison Service of the Leiden University Medical Center and were matched on age and BMD. A number of these control patients have been also reported in an earlier study (n = 12) (16). Control patients who reported fragility fractures or who had grade 2 or 3 morphometric vertebral fractures were excluded from the

**Figure 1**

Flowchart of patient recruitment.

study. A fragility fracture was defined as any low-energy fracture, excluding those of the hands, feet, and skull. Control patients with fractures after a high-energy trauma or grade 1 morphometric vertebral fractures were included in the study.

Both acromegaly and non-acromegaly control patients were excluded if they had a metabolic bone disease other than osteoporosis, any untreated endocrine disorder, severe liver insufficiency or chronic kidney disease stage IV or V, have had bilateral hip replacement, were currently using aromatase inhibitors or androgen deprivation therapy and were currently using or had used bisphosphonates, denosumab, selective estrogen receptor modulators (SERMs), strontium ranelate or recombinant PTH. Furthermore, patients were excluded if they had a localized infection of the tibia or were unable to provide informed consent. Current or past use of glucocorticoids was also an exclusion criterion with the exception of hydrocortisone supplementation for secondary adrenal insufficiency.

Methods

Medical history data including date of start of treatment, type of treatment (surgery, radiotherapy, medication use or combined), date of normalization of IGF-1, data on other pituitary function tests, menopausal status, previous or current medication use and fracture history were collected at time of inclusion in the study. Daily calcium intake was calculated and clinical risk factors for fracture as used in the FRAX algorithm were obtained from each patient.

Laboratory investigations

Serum calcium, phosphate, albumin and creatinine were measured using semi-automated techniques. Plasma intact PTH was measured by the immulite 2500 (Siemens Diagnostics) and serum 25-hydroxyvitamin D was measured using the 25-OH-vitamin D TOTAL assay (DiaSorin D.A./N.V.). Procollagen type 1 amino-terminal propeptide (P1NP) and β -Crosslaps were measured by an electrochemoluminescent immunoassay with a Modular Analytics E-170 system (Roche Diagnostics).

Serum IGF-1 levels (nmol/L) were measured using an immunometric technique on an Immulite 2500 system (Diagnostic Products Corporation). The intra-assay variations at mean plasma levels of 8 and 75 nmol/L were 5.0% and 7.5%, respectively. IGF-1 levels were expressed as SDS, using λ - μ - σ smoothed reference curves based on measurements in 906 healthy individuals(18, 19).

Serum GH levels were measured with a immunofluorometric assay (Siemens Diagnostics) calibrated against World Health Organization (WHO) National Institute for Biological Standards and Control (NIBSC) 2nd International Standard 98/574. Values were multiplied by 1.02.

Bone Mineral Density

BMD was measured at the lumbar vertebrae (L1-L4) and at the left and right femoral neck using Dual-energy X-ray Absorptiometry (DXA) (Hologic QDR Discovery A (Hologic, Bedford, MA, USA)) at time of indentation. NHANES III reference values compatible with reference values of the Dutch population were used to calculate T-scores. Normal bone mineral density, osteopenia or osteoporosis were diagnosed using WHO criteria.

Spinal radiographs

Conventional antero-posterior and lateral radiographs of the thoracic and lumbar spine were performed following standard protocols, at a focus-detector distance of 115 cm, with the detector centralized on Th7 for the thoracic spine and on L3 for the lumbar spine at time of inclusion. The semi-quantitative method of Genant was used to assess the presence and grading of vertebral fractures. Vertebral fractures were

scored as grade 1 (reduction in anterior, middle and/or posterior height between 20% and 25%), grade 2 (reduction in anterior, middle and/or posterior height between 25% and 40%) or grade 3 (reduction > 40% in anterior, middle and/or posterior height) (20), without a history of high energy trauma.

Radiographs were independently assessed by two of the authors (NA-D, HK). Consensus was reached in case of difference in assessment.

Impact microindentation

A Reference Point Indentation tool, the Osteoprobe[®], specifically designed for in vivo measurements of Bone Material Strength index (BMSi) in humans and large animals(21), was used as a surrogate to evaluate bone material properties(16). Impact microindentation is performed on the midshaft of the tibia by inserting a test probe in the skin until the bone surface is reached. By indenting the bone surface, the resistance of the bone tissue to fracture is evaluated; Fig 2. The first studies performed in humans with a previous version of the technique, have shown that postmenopausal women with osteoporotic fracture and with atypical femoral fracture have significantly higher indentation distance increase (IDI) than controls without fractures. Further studies using the impact microindentation technique demonstrated that patients with suspected deteriorated material properties such as patients with fragility fractures or patients with Type 2 Diabetes Mellitus had significantly lower BMSi values(13–16). The measurement site was defined as the mean distance between the distal apex of



Figure 2

Positioning of the test probe perpendicular to the bone surface.

the patella and medial malleolus. Local anaesthesia (Lidocaine 1%) was applied at the measurement site. The test probe was inserted at the site of interest after successful anaesthesia of the skin and periosteum and was pushed gently until it reached the bone surface. 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 “poorly performed” if the measurement probe slipped or if the subject moved his/her leg. At least five adequate measurements were obtained from each patient, followed by five additional measurements performed on a polymethylmethacrylate (PMMA) calibration phantom. The number of measurements ranged from 10 to 24. The resultant outcome BMSi was defined as 100 times the ratio of the harmonic mean indentation distance increase from impact into the PMMA calibration phantom divided by the indentation distance increase from impact into bone(21).

The intra-observer coefficient of variation (CV) was 2.2%. Ten subjects were measured twice on the same leg. The distance between the two measurement sites was 2 centimetres.

Statistical analysis

Analyses were performed using SPSS software for Windows (version 23.0; SPSS Inc., Chicago, IL, USA). Data are expressed as mean $\pm$ SD unless otherwise stated. Normality assumptions were checked by evaluation of normality plots and by inspection of histograms of residuals from miscellaneous regression models. Differences between groups were assessed using a two-sample t tests or Chi-square tests. Pearson/Spearman correlation coefficients were used to express correlations between IGF-1 levels, duration of disease remission and BMSi values. Analysis of variance models with BMSi as outcome variable adjusted for age, were used to compare BMSi values between patients and controls. Differences were considered to be significant at $p < 0.05$.

RESULTS

We included 48 patients with acromegaly and 44 controls who were comparable in gender, age, BMI and BMD at the lumbar spine and at the femoral neck; Table 1. The two groups were also comparable regarding the number of patients who had sustained a non-vertebral fracture, 11 (23%) in acromegaly patients and 11 (25%) in controls. There were more acromegaly patients with a vertebral deformity than controls [28(58%) vs. 7(16%); $p < 0.001$].

Table 1. Characteristics of patients with acromegaly and non-acromegaly controls

	Acromegaly (n = 48)	Control (n = 44)	p-value
Age (years)	60.2 ± 11.0	60.5 ± 8.5	0.849
Male/female	26/22	22/22	0.689
BMI (kg/m ²)	28.2 ± 4.7	26.6 ± 4.3	0.087
Fracture	11 (23%)	11 (25%)	0.815
Vertebral Fracture	28 (58%)	7 (16%)	< 0.001
- Grade 1	28 (58%)	7 (16%)	< 0.001
- Grade 2/3	11 (23%)	0	0.001
Smoking (%)	6 (13%)	8 (18%)	0.449
Alcohol use > 3IU/d (%)	5 (10%)	8 (18%)	0.304
Glucocorticoids (%)	8 (17%)	5 (11%)	0.466
PTH (pmol/L)	4.6 ± 2.8	3.3 ± 1.5	0.014
Calcium (mmol/L)	2.38 ± 0.11	2.39 ± 0.10	0.473
25-OH D (nmol/L)	67.3 ± 28.5	67.3 ± 27.6	0.944
Creatinine (μmol/L)	78.8 ± 14.3	76.1 ± 18.4	0.471
GH (mU/L)	4.0 ± 5.5	-	
IGF-1 (nmol/L)	19.5 ± 7.6	-	
IGF-1 SD	1.0 ± 1.3	-	
LS BMD (g/cm ²)	1.04 ± 0.21	1.03 ± 0.13	0.850
T-score LS	-0.3 ± 1.8	-0.3 ± 1.2	0.963
FN BMD (g/cm ²)	0.84 ± 0.16	0.80 ± 0.09	0.246
T-score FN	-0.6 ± 1.2	-0.8 ± 0.8	0.358

Values are expressed as mean ± SD.

BMI Body Mass Index; PTH parathyroid hormone; GH growth hormone; IGF-1 Insulin-like Growth Factor 1; SD standard deviation; LS lumbar spine; FN femoral neck; BMD Bone Mineral Density.

Reference range PTH (0.7–8.0 pmol/L); Calcium (2.15–2.55 mmol/L); 25-OH D (50–250 nmol/L); Creatinine (64–104 μmol/L for male, 49–90 μmol/L for female); GH (0.00–7.25 mU/L); IGF-1 (6.8–26.5 nmol/L).

In the acromegaly group, 27 patients (56%) were in remission after surgery, 7 of whom received additional radiotherapy. 21 (44%) patients were controlled with ongoing medical treatment with somatostatin analogs only (n = 14), Pegvisomant, a GH receptor antagonist, only (n = 3) or combination therapy (n = 4), 1 of whom had received radiotherapy, 16 of whom had surgery and 4 of whom had both. The mean duration of acromegaly remission was 16.1 years (range 0.5–37.8 years). There were 8 patients with type 2 diabetes mellitus, 3 of whom were conservatively treated and 5 received drug therapy (4 oral anti-diabetic agents, 1 insulin therapy), 11 with treated hypothyroidism, 10 with treated adrenal insufficiency and 9 with treated hypogonadism.

Bone Material Strength index

Patients with acromegaly had significantly lower BMSi values compared to controls (79.4 ± 0.7 vs. 83.2 ± 0.7 ; $p < 0.001$), also after adjustment for age (79.4 ± 0.7 vs. 83.2 ± 0.7 ; $p < 0.001$); Fig. 3. This finding remained if patients with type 2 diabetes mellitus were excluded (79.2 ± 0.8 vs. 83.2 ± 0.7 ; $p < 0.001$). Although numbers are small, there was no statistically significant difference in BMSi in patients with acromegaly regardless of the therapeutic intervention used to normalize IGF-1 levels (surgery: 81.1 ± 1.0 , surgery and radiotherapy: 77.9 ± 1.7 , medical treatment: 78.4 ± 1.0 ; $p = 0.114$).

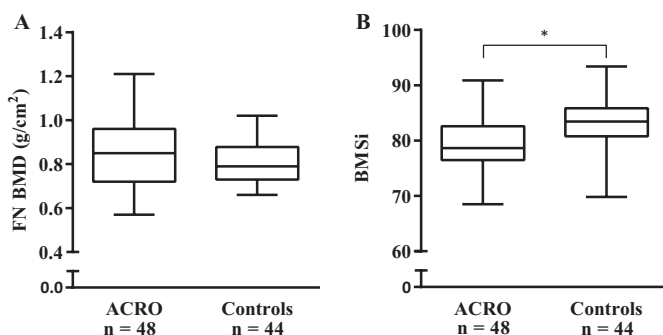


Figure 3

(A) Mean femoral neck Bone Mineral Density (FN BMD) and (B) Bone Material Strength index (BMSi) in patients with acromegaly and non-acromegaly controls.

Data are shown in box-whisker plots and statistical differences are displayed for BMSi. Boxes indicate median and inter-quartile range. Bars indicate minimum and maximum values.

* $p < 0.001$

In the group of patients with acromegaly, there was a significant relationship between BMSi and age ($r = 0.291$, $p = 0.045$). BMSi was not correlated with other clinical characteristics including BMI and duration of remission. There was also no relationship between BMSi and any biochemical parameter measured including parameters of calcium metabolism, serum levels of GH and IGF-1, gonadal hormone status or with BMD. There was no difference in BMSi values between male or female patients (79.5 ± 0.9 vs. 79.2 ± 1.0 ; $p = 0.804$), or between patients with or without a non-vertebral fracture (80.2 ± 1.4 vs. 79.1 ± 0.8 ; $p = 0.511$). There was no statistically significant difference in BMSi between patients with ($n = 28$) or without a vertebral fracture ($n = 20$); Supplemental Figure, also in the comparison between patients with Genant $\geq$ grade 2 vertebral fractures ($n = 11$) and patients with Genant $\leq$ grade 1 vertebral fractures ($n = 37$) (80.3 ± 1.4 vs. 79.1 ± 0.8 ; $p = 0.466$).

In the control group, there was a significant inverse relationship between BMSi and age ($r = -0.457$, $p = 0.002$), but no relationship between BMSi and any other parameter measured, including BMD.

DISCUSSION

To our knowledge, this is the first study examining tissue-level properties of cortical bone in patients with well-controlled acromegaly, previously shown to be at high risk for vertebral fractures independently of BMD. Our data show significantly lower bone material strength index (BMSi) values in patients with well-controlled acromegaly than in controls with comparable age and BMD. There was no difference in BMSi within the group of patients with acromegaly, in the presence or absence of vertebral fractures, or in the presence or absence of treated hypogonadism.

The reported prevalence of vertebral fractures in patients with acromegaly is high, ranging from 53 to 59%^(1, 2, 10). Longitudinal data also show that vertebral fractures continue to progress in controlled disease in patients both with and without previous vertebral fractures^(4, 5). This increased vertebral fracture risk in the presence of normal or high-normal BMD in patients with well-controlled acromegaly suggests that components other than bone mass may influence bone strength in these patients, such as bone microarchitecture or bone material properties. Indeed, histological findings from bone biopsies of the iliac crest performed in patients with active acromegaly confirm this suggestion by showing altered histomorphometric parameters of bone, particularly at cortical sites⁽⁶⁾. During active acromegaly, bone turnover is increased and this is associated with an increase in cortical bone mass. Trabecular bone mass remains stable although trabecular connections are lost due to the high bone turnover^(6–8). Further reported data have also shown an increase in the diameter of trabeculae, suggesting microarchitectural changes in the trabecular compartment of bone⁽²²⁾. These findings were further confirmed in a study using HRpQCT, which demonstrated altered microarchitecture of trabecular bone in eugonadal acromegalic patients compared to healthy controls⁽⁹⁾. The observations from the same study regarding the cortical compartment appear to contrast with the findings from our study, as cortical volumetric BMD was increased in active acromegalic patients. However, these patients were compared with patients with controlled acromegaly and our cohort consists of well-controlled acromegaly patients only.

A recent study in transgenic bGH mice demonstrated poor microarchitecture of both cortical and trabecular compartments of the tibia. This is an animal model with pathological elevated GH levels occurring in utero and in adult life. Although this mouse

model develops increased GH levels during skeletal development and is therefore not fully comparable with the manifestation of GH excess after epiphyseal closure in acromegaly, it may provide interesting observations on the effect of GH on the skeleton. The bGH mice were shown to have a significantly lower trabecular number and lower trabecular thickness than control mice, in the presence of overall larger bones and increased tibia length. bGH mice were also found to have increased cortical bone perimeter and cross-sectional area in the tibia, compared with control mice, despite lower cortical bone thickness(23). Cortical BMD was also significantly lower in the vertebrae of bGH mice compared to control mice. Vertebral trabecular BMD was comparable but trabecular thickness was decreased. Mechanical tests performed on the femora showed significantly lower mechanical properties, including ultimate stress and Young's modulus, in bGH mice compared to littermate controls. These results suggest that the cortical compartment in vertebrae is perhaps more affected than its trabecular compartment as a result of exposure to excess growth hormone. These data may provide some further understanding of our finding of a lower Bone Material Strength index, which mainly measures cortical bone properties, in patients with acromegaly with and without fractures.

The observation that BMSi of acromegaly patients with vertebral fractures was not statistically different from BMSi of acromegaly patients without vertebral fractures might be in keeping with previous suggestions that acromegaly affects not only the trabecular compartment but the cortical compartment as well. For instance, Vallassi *et al* reported that both cortical and trabecular volumetric BMD were reduced at the proximal femur in 35 acromegaly patients, regardless of gender, gonadal status, and disease activity(24). Recently, Maffezzoni and colleagues reported in 40 patients with acromegaly that those with vertebral fractures (n=15) had lower bone volume/trabecular volume ratio, greater mean trabecular separation, and higher cortical porosity compared to those without vertebral fractures (n=25)(11). However, as is also seen in our study, group sizes are small due to the rarity of the disease and preclude firm conclusions.

Impact microindentation is an emerging Reference Point Indentation technique that measures tissue-level properties of cortical bone in vivo at the mid-shaft of the tibia, thus reflecting tissue-level properties of cortical rather than trabecular bone. Although this technique appears as a promising alternative to assess bone fragility, data on fractures (including vertebral fractures) and Bone Material Strength index are relatively limited. A preliminary report of postmenopausal women with (49% vertebral fracture, 51% non-vertebral fracture) or without fractures found lower BMSi values in fracture patients and a significant inverse relationship between BMSi and grade of vertebral fracture(25), whereas a recent study in elderly community-dwelling women showed

no relationship between prevalent vertebral fractures and BMSi(26). Within our own cohort of patients with low bone mass and fragility fractures, we demonstrated that BMSi was low irrespective of the type of fracture: vertebral, non-vertebral or a combination of the two fracture types(27). The difference in BMSi between patient groups (including fracture patients) and controls in reported literature is variable from 4.5% up to 9.2%(15, 16, 25, 28). Although our findings are in keeping with those from other groups, the predictive value remains to be determined.

This study has strengths as well as limitations. A major strength of the study is having had access to a large cohort of well-characterized patients with acromegaly, all hormonally and metabolically well controlled. A limitation of the study is the cross-sectional design. It remains to be established whether BMSi is a predictor for vertebral fractures in acromegaly patients in prospective studies. Also, we were only able to obtain areal BMD data from all patients but not volumetric BMD in any. GH significantly increases the size of the skeleton in patients with acromegaly and areal BMD does not adjust for the size of bone. Although acromegaly is associated with comorbidities such as type 2 diabetes mellitus and hypogonadism, which in itself may contribute to bone fragility, all endocrine deficiencies were adequately supplemented and diabetes well controlled by their treating physician as required. Lastly, patients with a very high fracture risk could not be included in this study, because these patients were currently or had been previously treated with bone modifying agents.

Findings from our study add to the accumulating evidence for the association of acromegaly with structural changes of bone, also after adequate control of growth hormone production. This is in keeping with findings from two recent studies that reported a decrease in trabecular bone score (TBS) in the presence of an increase in lumbar spine BMD after successful treatment of acromegaly(29), and a decrease in cortical and trabecular volumetric BMD measured at the femur, independently of acromegaly disease status(24).

In keeping with our previous observation, we found a strong inverse relationship between BMSi and age in our control group. In contrast, we found a positive relationship between BMSi and age in patients with acromegaly. A possible explanation of this contrasting finding is that older patients had longer remission periods and the skeleton may have had more time to recover from the effects of high GH levels. This may suggest that acromegaly patients with more recent exposure of high GH levels (the young patients with acromegaly) have lower BMSi values than patients with acromegaly with longer duration of remission. However, we did not find a relationship between BMSi and the duration of remission in our series ($r=0.220$, $p=0.133$). There was no difference in BMSi values between men and women in the group of acromegaly

patients and in the control group. Although skeletal health has been shown to differ between the sexes, this observation is in keeping with other data on BMSi previously reported by our group(16) and another group(30), and partly in keeping with a study that investigated male and female HIV patients and controls and observed lower BMSi values in female HIV patients compared to male patients but comparable BMSi values between male and female controls(31).

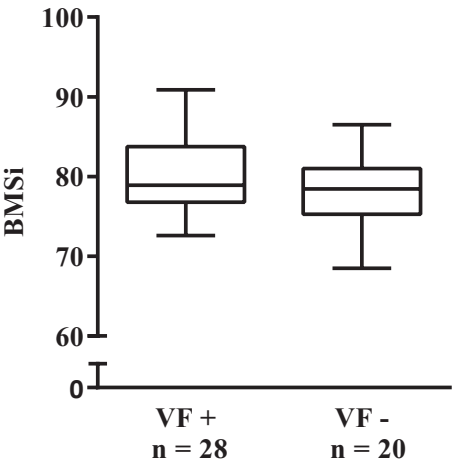
In conclusion, we demonstrate that BMSi is significantly lower in patients with well-controlled acromegaly compared to controls. These data indicate that tissue-level properties of cortical bone remain significantly altered after cessation of long-term exposure to pathologically high GH and IGF-1 levels, probably contributing to the previously demonstrated ongoing increased risk for vertebral fractures. Future prospective studies addressing the skeletal complications of acromegaly should incorporate the study of microarchitecture and material properties of bone in addition to bone mass measurements.

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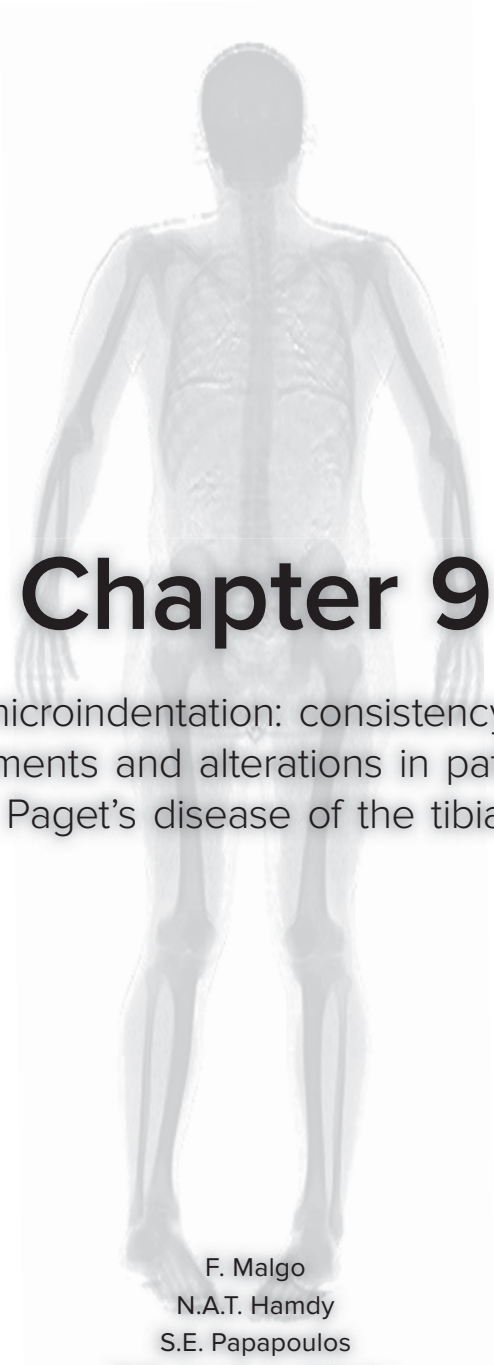
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Supplemental Figure

Bone Material Strength index (BMSi) in patients with acromegaly, with (VF+) and without (VF-) vertebral fractures. 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.



Chapter 9

Impact microindentation: consistency of serial measurements and alterations in patients with Paget's disease of the tibia

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ABSTRACT

Impact microindentation is a new technique for the *in vivo* measurement of tissue-level properties of cortical bone in humans. To address issues related to the proper application of IMI in clinical practice and to directly examine cortical bone properties in patients with tibia pathology, we studied 11 subjects without tibia pathology and 9 patients with Paget's disease of the tibia in biochemical remission after bisphosphonate treatment. Serial indentations in the tibiae of both legs were performed in all subjects by a single operator until 10 adequate measurements were obtained in each tibia. In patients without Paget's disease [7 men and 4 women; mean age 61.9 years (range 51–72 years)], there was no difference in mean Bone Material Strength index (BMSi) between the dominant and non-dominant leg (82.1 ± 1.3 and 81.4 ± 1.3 , respectively; $p = 0.606$). In each individual subject studied, sequential indentations in both legs showed no trends for higher or lower values with time. The standard deviation of unnormalized Bone Material Strength (BMSu) was also comparable between the dominant and non-dominant tibia (5.3 and 4.5, respectively; $p = 0.657$). In patients with Paget's disease [4 men and 5 women; mean age 69.5 years (range 55–87 years)], mean BMSi of the Pagetic tibia was lower, albeit non-significantly, than that of the contralateral non-affected tibia (74.7 ± 1.7 and 78.7 ± 1.3 , respectively; $p = 0.120$). In contrast to subjects without Paget's disease, the SD of adequate BMSu values was significantly larger in the Pagetic tibia compared to that of the non-Pagetic tibia (7.6 vs. 5.0, respectively, $p = 0.008$). These results highlight the consistency of serial IMI measurements as performed by a single operator in the presence as well as absence of tibia pathology and illustrate that the method is able to capture alterations of tissue-level cortical bone properties in patients with Paget's disease of the tibia.

INTRODUCTION

Impact microindentation (IMI) is a new Reference Point Indentation technique that measures tissue-level properties of cortical bone in humans *in vivo* at the mid-shaft of the tibia. The tool used to perform IMI is a hand-held device (OsteoProbe) that imparts a single impact load to the bone surface. The methodology and clinical value of the method have been recently reviewed ^(1–3). In the course of the investigation, repeated indentations are performed on the tibia until a minimum number of adequate measurements is obtained. The manufacturer of the device does not provide an algorithm to automatically flag inadequate measurements, the evaluation of which is left to the judgement of the operator during the procedure. To our knowledge, the variance of measurements obtained in an individual subject by sequential adequate indentations has not been previously reported. Moreover, whereas the non-dominant leg is recommended as the preferred investigation site ⁽³⁾, data supporting this recommendation are not available. An important, clinically relevant question is whether the application of IMI is informative and reliable in patients with tibia pathology, particularly as pathology at this site has so far been considered as an exclusion criterion. IMI measurements may indeed provide valuable information not only on the tissue-level properties of the diseased tibia, but also on the performance of the technique in general.



Figure 1

Radiographs of tibiae of two patients with Paget's disease of bone included in the present study with characteristic changes.

To address these questions and to obtain more insight into the value of IMI in clinical practice, we examined tissue-level properties of cortical bone of both legs in individuals without tibia pathology and in patients with Paget's disease of the tibia. Localizations of Pagetic lesions in the tibia can be found in the original description of the disease by Sir James Paget in 1887, ⁽⁴⁾ and we have previously reported that lesions of the tibia comprised 7% of all Pagetic lesions in 180 consecutive patients from our cohort of patients with Paget's disease of bone ⁽⁵⁾. Paget's disease of the tibia has characteristic radiologic features such as cortical thickening and abnormal bone texture (Figure 1) and may be associated with significant deformity, pain and increased risk of stress and complete fractures ⁽⁶⁾.

PATIENTS AND METHODS

Impact microindentation was performed in all subjects by a single operator (FM) in the Out-Patient Clinic of the Center for Bone Quality of the Leiden University Medical Center (LUMC). The group of patients without tibia pathology included in this study consisted of consecutive patients attending our Out-Patient Clinic who participated in our studies of tissue-level properties of cortical bone in patients with and without fractures and with well-controlled acromegaly and who consented to having further IMI measurements undertaken in both legs. Details of the characteristics and selection of these patients have been previously published ^(7,8). Exclusion criteria included localized infection of the tibia, metabolic bone disease other than osteoporosis, any untreated endocrine disorder, bilateral hip replacement, severe liver insufficiency, chronic kidney disease stage IV or V, and current or past use of glucocorticoids with the exception of hydrocortisone supplementation for secondary adrenal insufficiency in patients with acromegaly and inability to provide informed consent. All patients with radiologically confirmed Paget's disease of the tibia who were followed regularly in our Center and were in remission after treatment with bisphosphonates were also studied. The study was approved by the Medical Ethics Committee of the LUMC and all participants provided written informed consent.

Data on age, gender, height and weight, history of fractures and use of medications (current and previous) were documented in all patients included in the study. In subjects without Paget's disease, the presence of morphometric vertebral fractures was examined by spine radiographs and Bone Mineral Density was measured by DXA. In patients with Paget's disease the date of diagnosis of the disease and dates, type and number of bisphosphonate treatments were recorded and radiographs of the tibiae were reviewed to confirm the presence of a Pagetic lesion of the tibia.

Laboratory investigations

Blood was collected and serum measured for calcium, phosphate, albumin and creatinine using semi-automated techniques, for alkaline phosphatase (ALP) using a fully-automated P800 modulator system (Roche Diagnostics), for procollagen type 1 amino-terminal propeptide (P1NP) using an electrochemoluminescent immunoassay with a Modular Analytics E-170 system (Roche Diagnostics) and for 25-hydroxyvitamin D using the 25-OH-vitamin D TOTAL assay (DiaSorin D.A./N.V.). Plasma was measured for intact PTH using the Immulite 2500 assay (Siemens Diagnostics).

Impact microindentation

Bone Material Strength index (BMSi) was measured in all subjects by IMI applied to the mid-shaft of the tibia by a single operator (FM) using a hand-held microindenter (OsteoProbe® RUO, Active Life Scientific, CA, USA), as previously described and recommended by a group of experts ⁽³⁾. 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. Indentations of both tibiae were performed in a single session without repositioning of the patient. The measurement site was defined as the midpoint of the distance between the distal apex of the patella and medial malleolus. The operator ensured that the test probe was placed perpendicularly to the bone surface and classified the measurements as “well performed”, “adequate” or “poorly performed” after the indentation and before checking the computer display according to the following criteria: “well performed” when the operator judged that the test probe was exactly perpendicular to the bone surface; “adequate” when the test probe was within acceptable deviation from the bone surface ⁽⁹⁾; “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 or failure to place the device perpendicularly to the bone surface and are excluded from the analysis. In the present study the operator continued the protocol until 10 adequate measurements were obtained. Five additional measurements were subsequently performed on a calibration phantom, a cube of polymethylmethacrylate (PMMA), firmly secured in a holder and placed on a stable surface. BMSi is calculated as 100 times the harmonic mean of the indentation distance increase from impact into the PMMA material divided by the indentation distance increase from impact into the bone ⁽³⁾. Another parameter, unnormalized BMS, has been used in the original description of the method to describe the effects of instrument variables on measurements ⁽⁹⁾. The authors of this publication concluded that unnormalized BMS directly obtained by measurement of individual sequential indentations, is more suitable for the analysis of variability of the procedure than BMSi, which is the final result of all sequential measurements in a single patient after correction with the PMMA calibra-

tion phantom. Normalization of the measured values to the calibration phantom, as done in the standard calculation of BMSi, would remain relatively consistent when a variable, such as probe sharpness or indentation angle, is changed. Unnormalized BMS is defined as 100 times the ratio of the ideal indentation distance increase from the impact into PMMA (150 μm) divided by the indentation distance increase from the impact into the bone sample. We used this parameter for the calculation of variability of sequential measurements in the same subject and we arbitrarily defined it BMSu as opposed to BMSi.

The intra-observer coefficient of variation (CV) of the technique was calculated to be 2.2% in a previous study of patients with low bone mass with or without fractures by measuring BMSi twice in the right leg according to the above mentioned protocol with the second investigation performed 2 centimetres below the first measurement site in 10 subjects ⁽¹⁰⁾. Mean $\pm$ SD BMSi of the two measurements was 75.9 ± 5.7 and 76.6 ± 4.9 , respectively.

Statistical analysis

Indentations judged “poorly performed” by the operator were not included in the calculation of the final BMSi or in the variation analyses. Results are reported as mean $\pm$ SE unless otherwise stated. Between-group differences in baseline characteristics were assessed using a Student t test, a chi-square test, or a Mann-Whitney U test for non-normally distributed variables. Pearson’s and Spearman’s correlations were used to assess correlations between BMSi and patients’ parameters that were normally and not normally distributed. A paired t-test was used to compare BMSi values between both tibiae. A Wilcoxon Signed Ranks Test was used to compare the standard deviation of the ten adequate BMSu values between both tibiae. Differences were considered to be significant at $p < 0.05$. All analyses were performed using SPSS software for Windows (version 23.0; SPSS Inc., Chicago, IL, USA). Scatterplots were constructed with Graphpad Prism (version 7.0; Graphpad Software Inc., La Jolla, CA, USA); BMSu values were plotted in the sequence they were obtained from the first to the last.

RESULTS

We studied 11 consecutively presenting individuals (7 men) aged 61.9 years (range 51 to 72 years) and BMI 27.3 ± 1.7 , without pathology of the lower extremities and who consented to bilateral IMI measurements. Four had osteoporosis, 5 had osteopenia and 2 had normal Bone Mineral Density. Two patients with osteoporosis had a history of a clinical vertebral fracture and one with osteopenia of a distal radius fracture. Five patients from the acromegaly cohort had morphometric vertebral fractures. In

all subjects studied biochemical parameters of bone and mineral metabolism were within the normal laboratory reference ranges (not shown). Nine patients (4 men) with Paget's disease of the tibia with a mean age of 69.5 years (range 55 to 87 years), and BMI 27.5 ± 1.1 , who were in biochemical remission for at least one year after treatment with bisphosphonates (median 5.5 years, range 1–30 years) were also studied. At the time of the investigation mean serum alkaline phosphatase activity was 76.7 ± 5.7 IU/l (reference range 40–120 IU/l) and serum P1NP was 41.8 ± 4.6 ng/ml (reference range < 65 ng/ml).

Impact microindentation in subjects without tibia pathology

In subjects without Paget's disease a mean of 12 indentations (range 11–15) were performed in the dominant leg and 12 indentations (range 10–14) in the non-dominant leg in order to obtain 10 adequate measurements in each tibia of all tested individuals. Inadequate measurements were evenly spread during the procedure with the exception of the first indentation that was classified as “poorly performed” by the operator in 10 of the 22 measurements (7/14 and 3/8 measurements in acromegaly and remaining patients, respectively). Mean BMSi of the dominant leg was not different from that of the non-dominant leg (82.1 ± 1.3 and 81.4 ± 1.3 , respectively; $p = 0.606$). Individual adequate measurements of the same tibia generally showed minimal variations (Figure 2A), but larger variations were also observed (Figure 2B), which did not in any case affect the

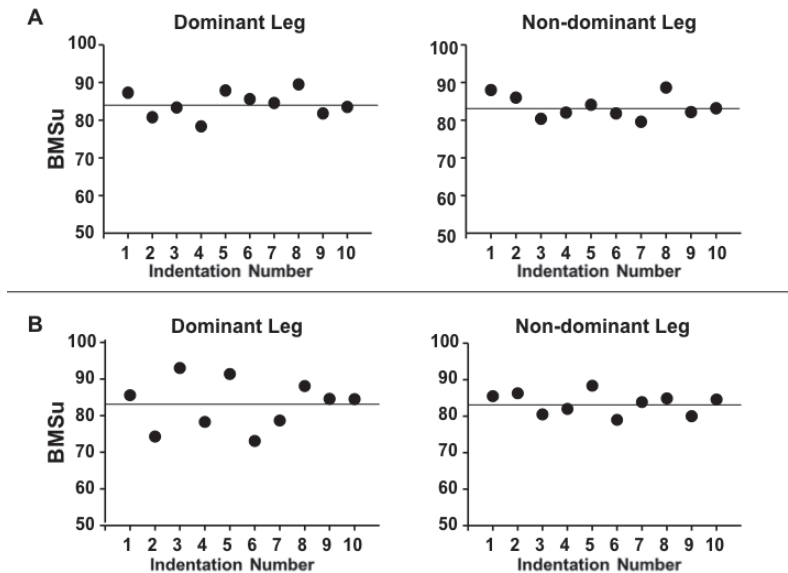


Figure 2

Scatterplot of 10 sequential adequate indentations of both legs in 2 subjects (A and B) without tibia pathology.

mean BMSi value. These larger variations were not operator-dependent and bore no relation to the sequence timing of the indentation. This is illustrated in Figure 3 by the lack of a trend towards lower or higher values or alterations in the magnitude of SDs of the mean BMSu of sequential adequate indentations. Mean SDs of BMSu of the dominant and non-dominant legs were overall not different (5.3 and 4.5, respectively; $p=0.657$). Similarly, there was no difference in mean SD of BMSu of the right and left leg (5.1 and 4.7, respectively; $p=0.657$). There was no difference in BMSi values after 5 or 10 adequate measurements in either investigated leg (82.7 ± 1.2 vs. 82.1 ± 1.3 and 81.1 ± 1.3 vs. 81.4 ± 1.3 for the dominant and non-dominant leg, respectively).

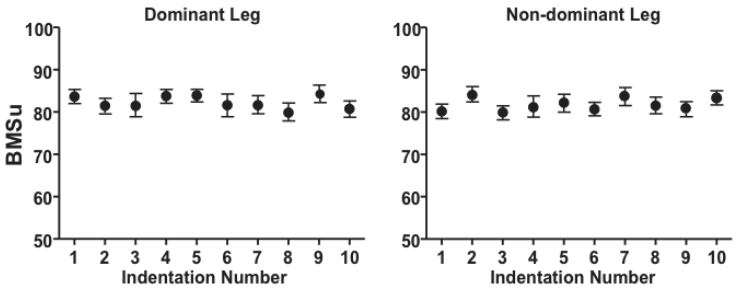
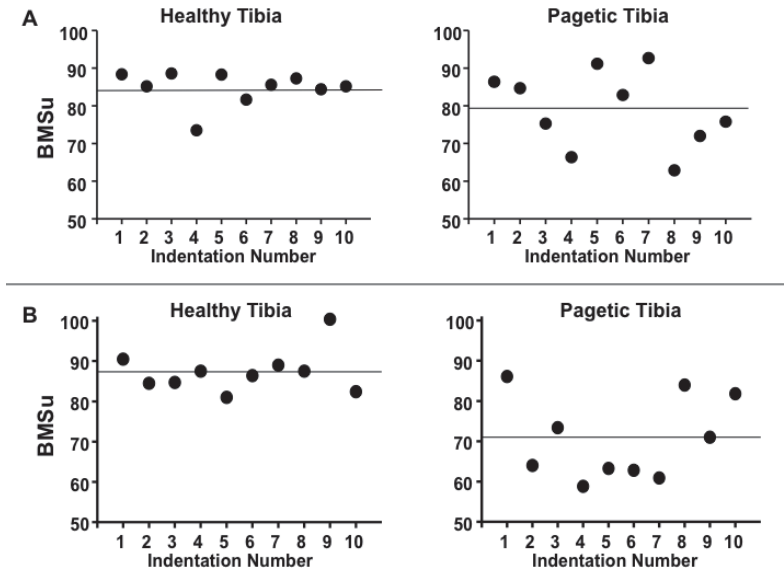


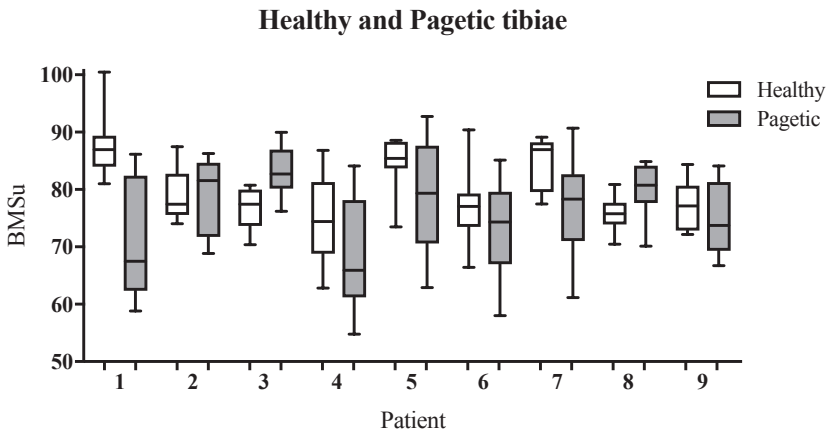
Figure 3
Scatterplot of standard deviations of mean unnormalized Bone Material Strength (BMSu) of sequential adequate indentations.

Impact microindentation in patients with Paget's disease of the tibia

In patients with Paget's disease a mean of 11 indentations (range 10–12) were performed in the affected tibiae and 12 indentations (range 10–16) were performed in the non-Paget tibia in order to obtain 10 adequate measurements of each tibia. Mean BMSi of Pagetic bone was lower, albeit non-significantly, than that of the contralateral non-affected bone (74.7 ± 1.7 and 78.7 ± 1.3 , respectively; $p=0.120$). In contrast to the observations in subjects without Paget's disease, the variability of measured BMSu values of the Pagetic tibia was greater than that of the contralateral tibia (Figures 4 and 5). This was confirmed by the significant difference in mean SDs of BMSu measurements between Pagetic and non-Paget bones (7.6 vs. 5.0, respectively, $p=0.008$). In none of the patients with Paget's disease was the SD of BMSu larger in the unaffected bone compared to the affected one.

**Figure 4**

Scatterplot of 10 sequential adequate indentations of the healthy and of the affected tibia in 2 patients (A and B) with Paget's disease of bone.

**Figure 5**

Box-plots of unnormalized Bone Material Strength (BMSu) values of healthy and affected tibiae of all patients with Paget's disease of bone included in the study.

DISCUSSION

The *in vivo* measurement of IMI in the tibia of humans using the OsteoProbe is a new technique that provides information about tissue-level material properties of cortical bone. Although the relationship between measured properties of bone and its traditional mechanical properties still needs to be determined, clinical studies have demonstrated that the method could differentiate patient groups at increased risk for fracture from control groups independently of BMD values, suggesting that the method measures aspects of bone fragility not captured by BMD ^(7,11–13). In the present study we report for the first time serial indentations of both legs in all tested subjects to address issues related to the proper application of this technique in clinical practice and to directly examine properties of cortical bone in patients with Paget's disease of the tibia compared to cortical bone of non-pathologic tibiae.

Results obtained by *in vivo*, non-automated methods of investigation are subject to variations that depend on the operator, on the performance of the procedure and on underlying individual biological variance. Results obtained using the OsteoProbe may be affected by all these factors and adequate evaluation of these factors may help in explaining some discrepancies in reported results between different groups that raised questions regarding the value of this method in the Clinic ⁽²⁾.

The IMI method used in the present study does not automatically exclude inadequate measurements, a task which is left to the judgement of the operator. This has been an issue of critique, particularly as there has been no specific reference to flagged results in most reported studies so far. Our data from this study demonstrate that in the hands of an experienced operator, who applied exclusion of inadequate indentations without knowledge of the result, up to 16 indentations was needed in order to obtain 10 adequate measurements. Although we have no data to compare the performance of different operators, our previous results with a single operator showing minimal intra-observer variation are clearly at odds with studies reporting results obtained by different operators, which showed large and significant variations in measured BMSi values requiring statistical adjustment of the results ⁽¹⁴⁾. This confirms the notion that an experienced operator is a *sine qua non* for the successful clinical application of the technique. If this is not feasible, additional operators should undergo a period of training with continuous comparison of their performance with that of an experienced operator. Relevant to this issue is the variable number of indentations required for the final results in different studies that ranged from 5 adequate to 15 in total ^(7,11,14–16). In the recent technical report of the application of OsteoProbe in human studies, 5 adequate indentations are recommended to be performed in each tested subject ⁽³⁾.

We found no difference in final BMSi values between 5 and 10 adequate indentations, a result that is in agreement with the recommendation. However, more than 5 indentations may be required when an intrinsic, operator-independent, variability in values is expected. The exclusion of the result of the first indentation routinely and independently of the operator's evaluation, as used by some investigators, has also been recommended ⁽³⁾. In our study flagged measurements were evenly distributed among sequential indentations for up to 16 indentations with the exception of the first indentation that had to be flagged by the operator in 10 of the 22 measurements in subjects without Paget's disease. This high number of inadequate first indentations justifies the recommendation of routinely excluding the first indentation value from the measurements. On the other hand, a remarkable consistency of sequential results and minimal variability in mean values was observed indicating a robust method. Although variability may be greater in individual subjects this did not seem to affect the final outcome. These findings, together with the overlap of values between tested control and patient groups in reported studies, suggest that whereas the method can currently provide valid information in groups of individuals, it cannot be recommended as yet for drawing firm conclusions about tissue material properties of cortical bone in individual patients. Finally, we show here that in the absence of tibia pathology, there is no difference in measured BMSi between the two legs. Although the harmonized use of the non-dominant leg is recommended for consistency of data acquisition and for comparison of outcomes obtained with the use of the OsteoProbe between different centers ⁽³⁾, our bilaterally obtained data do suggest that the investigator's choice of the most suitable leg for testing may be allowed as this does not compromise the outcome of the procedure.

Taken at face value, our first ever reported results of impact microindentation in patients with Paget's disease of the tibia unexpectedly showing no significant decrease in BMSi in the affected compared to the normal tibia may seem surprising and perhaps disappointing about the value of IMI in discerning abnormal tissue level properties of cortical bone in Pagetic tibiae ⁽¹⁷⁾. However, a caveat in the interpretation of these results as well as of the absence of complications of the procedure in pathological tibiae, may be due to the small number of patients studied, limiting the power of the study to detect significant differences between healthy and pathological bone or to capture complications. It is also of note that all nine patients with Paget's disease were in biochemical remission for longer than one year following treatment with bisphosphonates and none had sustained a stress or complete fracture of the tibia or had been subjected to a corrective surgical procedure. Whereas the finding of no significant BMSi difference between pathological and healthy tibiae in patients with Paget's disease in our study may well have been related to bisphosphonate treatment

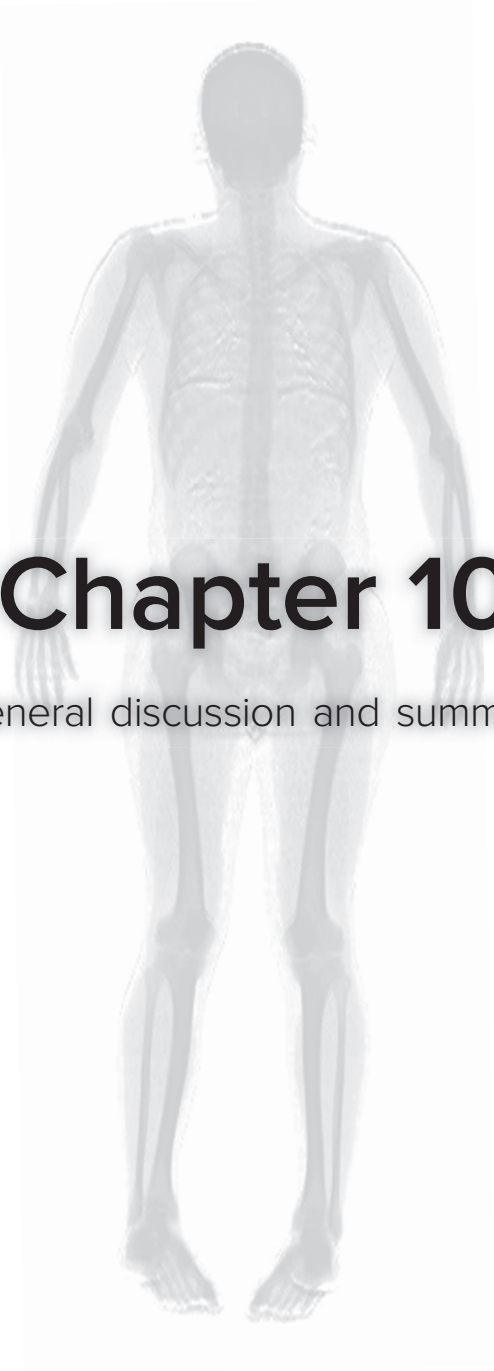
and long-term remission of the disease, our data cannot address this assumption, as we did not investigate treatment-naïve patients. Interestingly, Mellibovsky et al recently reported that risedronate treatment given for 20 weeks to patients treated with glucocorticoids significantly improved BMSi before any measurable change in BMD could be observed ⁽¹⁵⁾.

The most important novel finding in our study was the wide variability of adequate measurements of BMSu of the affected Pagetic tibia indicating a heterogeneity of tissue properties associated with the bone pathology of the disease. In this disorder, the localized accelerated bone remodeling results in the deposition of a disorganized mosaic of woven and lamellar bone leading to heterogeneous structural changes at affected skeletal sites. The BMSu values we recorded were very variable: within or higher than the range of values of the contralateral non-Pagetic tibia, interspersed with very low measured values. The variability of these measurements reveals a new, previously unreported, heterogeneous pattern of tissue level properties of pathological bone which is not due to methodological inadequacies and cannot be readily explained. This finding raises, in addition, the question whether variability of adequately performed indentations in individual subjects may enable the identification of aspects of biological variance of bone properties which are not captured by mean BMSi values. This issue warrants further investigation in appropriately selected patients or groups of patients.

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Chapter 10

General discussion and summary

INTRODUCTION

Fragility fractures are common and their incidence increases with age. These fractures are associated with increased morbidity and mortality and with a significant increase in social and economical burden. The presence of a fragility fracture is also a strong predictor for future fractures. Osteoporosis represents the main cause for fragility fractures and there has been a significant worldwide trend to establish fracture liaison services (FLS) in order to screen patients who have recently sustained a fracture for the presence of low bone mass using DXA. An FLS offers a multidisciplinary approach to identify patients at high risk for future fractures and to start treatment with anti-osteoporosis agents when required. A number of initiatives such as the global campaign “Capture the Fracture” of the International Osteoporosis Foundation and recommendations issued by various national and international guidelines have driven the initiation and implementation of these services worldwide, including in the Netherlands, aiming at the secondary prevention of fragility fractures in patients who have recently sustained a fracture. It has been shown that the use of these services has already led to a reduction in mortality and in the incidence of new fractures, and that their use has been proven to be cost-effective. The results of our experience with our own FLS in the Leiden University Medical Center (LUMC) are discussed in **Chapters 2 to 4**. The LUMC is a teaching hospital with its FLS serving the emergency and trauma center for primary fracture care for patients who have sustained an acute fracture. All fracture patients are identified by a dedicated FLS coördinator at the outpatient clinic of the Departments of Traumatology and Orthopaedic Surgery. Patients aged 50 years or older are invited to be screened for the presence of osteoporosis and for the risk of future fractures. Upon consent, the FLS coördinator immediately initiates the screening programme which consists of collection of data on clinical risk factors for fracture, performing laboratory investigations and of a Bone Mineral Density (BMD) measurement including a Vertebral Fracture Assessment (VFA) in the same sitting (Figure 1). Using this approach has succeeded in initially capturing more than 50% of all patients referred with a recent fracture for screening for increased fracture risk and when necessary start of treatment at the start of the FLS in 2012. After several improvements such as combining the appointments for both BMD measurements and laboratory investigations on the same day as that of the patients’ regular follow-up visit to the outpatient clinic of Traumatology or Orthopaedic Surgery, the rate of patients’ referrals to the FLS had risen to 95% in 2016.

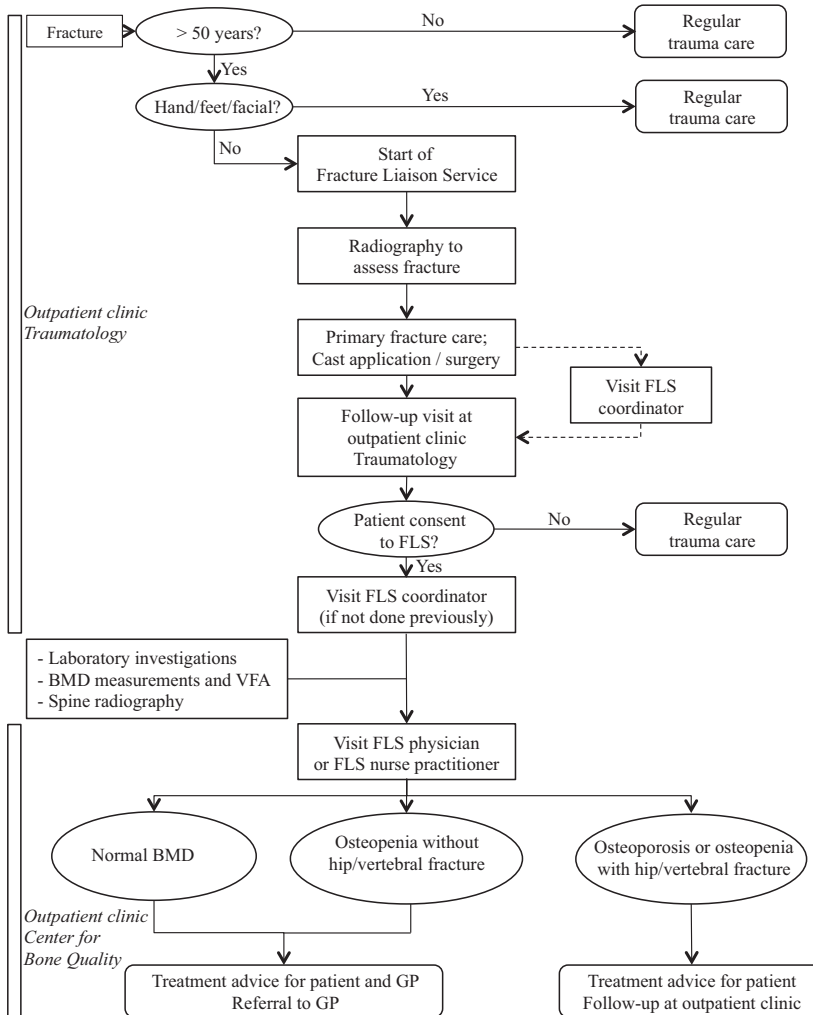


Figure 1
Flowchart Fracture Liaison Service

PART 1: THE FRACTURE LIAISON SERVICE

In this thesis, **Chapter 2**, **Chapter 3** and **Chapter 4** discuss the results of data collected from all patients who were referred to our FLS from June 2012 to June 2014 for screening for osteoporosis and for increased fracture risk.

Chapter 2 reports on the prevalence of underlying secondary factors for increased bone fragility in 686 patients who had recently sustained a fragility fracture. Data from this study demonstrate a high prevalence of underlying secondary factors in patients who had recently sustained a fracture, independently of whether they had a normal

BMD, osteopenia or osteoporosis (47, 57 and 62%, respectively). A significant negative relationship was identified between the cumulative number of underlying factors and BMD measurements. Previous studies had already reported a high number of underlying factors in patients with recent fractures, although mainly focusing on patients with osteoporosis. Our data complements data obtained from these studies in providing evidence for the importance of these underlying factors, independently of BMD status. A clinically relevant observation is that almost half of all underlying factors were potentially reversible by adopting specific lifestyle changes or by initiating medical treatment suggesting that the increase in bone fragility may be decreased upon reversal of these factors. As underlying factors for osteoporosis are equally prevalent in patients with recent fractures independently of BMD, this study further emphasizes that screening for these factors should be performed in all patients with fragility fractures and not just in those with osteoporosis.

Chapter 3 describes a retrospective study assessing the value of low-radiation VFA compared to conventional radiography of the thoracic and lumbar spine in identifying vertebral fractures in the FLS cohort of patients described in **Chapter 2**. Only patients with data available for both VFA and conventional spine radiography were included in this study. VFA is an additional tool available for use on DXA machines for the acquisition of lateral images of the thoracic and lumbar vertebrae in the same session at which BMD is measured. In our study, the sensitivity of low-radiation VFA to detect a patient with a vertebral fracture grade 2 or greater was 0.77 and the specificity was 0.80. This means that using VFA a vertebral fracture $\geq$ grade 2 was missed in 23% of patients. As these numbers were not in keeping with data from current literature on VFA, we went on to perform a systematic review and a meta-analysis of all published data on the value of Vertebral Fracture Assessment compared to conventional radiography in patients at risk for osteoporosis. Our systematic review covered 16 studies on the value of VFA including a total of 3238 patients. Our review shows that these studies were heterogeneous, with variable patient inclusion criteria, which have led to considerable variability in sensitivity (47–99%) and specificity (74–100%) between studies. Data from our meta-analysis demonstrated a sensitivity of 0.82 (95% CI, 0.75–0.87) and a specificity of 0.99 (95% CI, 0.98–1.00) for the ability of VFA to detect vertebral fractures grade 1 or more. Findings from our retrospective study were thus not in keeping with those obtained from our systematic review and meta-analysis. A possible explanation for this discrepancy in findings may be not only the different inclusion criteria, but also the use of different software and hardware to obtain VFA data. Based on this, we recommend that individual centers intending to use VFA should not replace conventional spinal radiography with VFA before the ability of their centers' VFA to detect patients with vertebral fractures has been investigated and established.

Bone turnover markers (BTMs) are not commonly used outside research settings in the follow-up of patients treated for osteoporosis although these markers can be easily measured in blood samples. However, data obtained from clinical trials have shown that BTMs may be potentially used to monitor response to treatment with anti-osteoporosis drugs as serum levels of these markers have been shown to be inversely related with changes in BMD and with anti-fracture efficacy. In **Chapter 4**, we assessed the longitudinal pattern of change in the bone turnover markers P1NP and Ctx after 3 and 12 months of treatment with antiresorptive drugs, and the relationship between these markers and BMD after 12 months of treatment in FLS patients who have recently sustained a fracture. Our results show that changes in P1NP levels after 3 and 12 months of treatment were significantly inversely related with changes in BMD at the lumbar spine after 12 months of treatment, although only the relationship between P1NP and lumbar spine BMD after 12 months persisted after adjustment for time after fracture. Changes in Ctx levels after 12 months of treatment were also significantly inversely related with BMD changes after 12 months of treatment after adjustment for time after fracture. We also observed a relationship between baseline P1NP values and the change in lumbar spine BMD at 12 months of treatment. Data from this study suggest that changes in bone turnover markers may be used early in follow-up of treatment to predict the effect of antiresorptive treatment on BMD in the long term. Whether early changes in BTMs are related to a decrease of future fracture risk remains to be established in large clinical follow-up studies.

PART 2: IMPACT MICROINDENTATION IN THE EVALUATION OF BONE FRAGILITY

Chapters 6 to 9 describe the results of our clinical studies performed using the impact microindentation technique (IMI), a new technique that measures tissue-level properties of cortical bone in humans *in vivo*. **Chapter 5** provides a general introduction and an up-to-date review of all published studies using the IMI technique.

In **Chapter 6**, we evaluated the relationship between Bone Material Strength index (BMSi) and fracture in 90 patients with low bone mass who were attending our FLS or the outpatient clinic of the Center for Bone Quality. We observed that patients who had sustained a fragility fracture had significantly lower BMSi values compared with patients with no fractures, despite similar BMD values between fracture and non-fracture patients. We also demonstrated a strong and significant inverse relationship between BMSi and age. These data suggest that tissue-level properties of cortical bone are

altered in patients who have sustained a fragility fracture, and that IMI captures certain elements contributing to bone fragility independently of BMD.

After publication of the results from our study described in **Chapter 6**, Duarte Sosa and colleagues published data from a further study showing significantly lower BMSi values in 66 postmenopausal women compared to 66 controls in addition to a significant relationship between BMSi and the severity of vertebral fractures. In contrast, a study from Rudang and colleagues found no relationship between BMSi and the presence of fractures, including vertebral fractures. To further investigate this discrepancy in data between studies, we evaluated the relationship between BMSi and different types of fragility fractures in our own cohort of patients with fragility fractures, the results of which are described in **Chapter 7**. In this study, we demonstrate that BMSi values were low in the presence of a fracture, irrespective of whether this was a vertebral or a non-vertebral fracture. We also found that BMSi values of patients with vertebral fractures were comparable with those of patients with non-vertebral fractures in addition to a vertebral fracture, suggesting that BMSi may reflect general bone fragility rather than just cortical bone fragility.

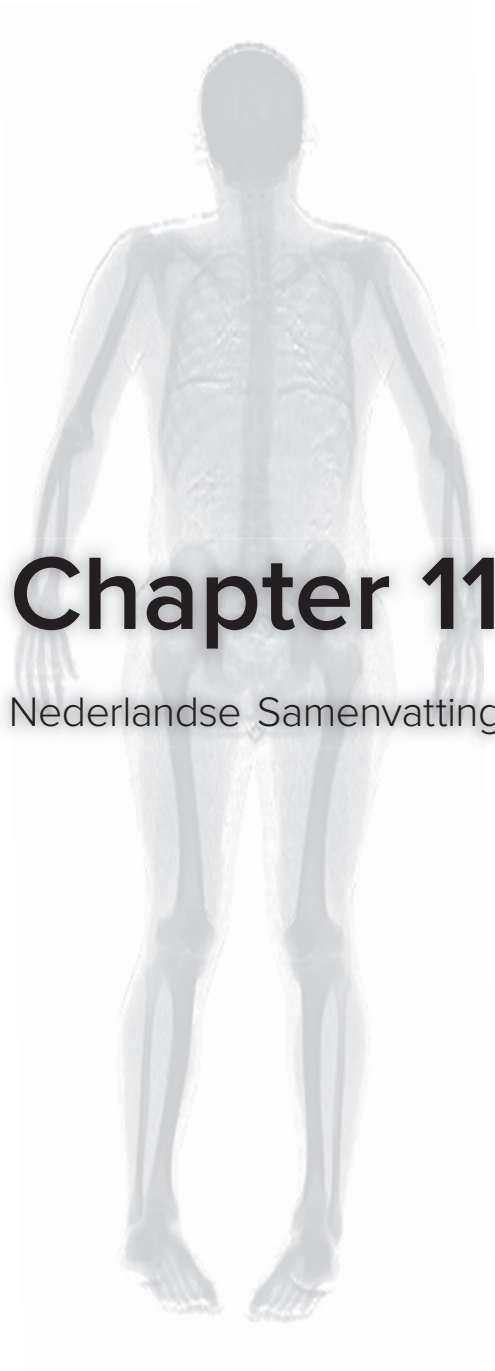
As a proof of concept, we evaluated tissue-level properties of bone in a cohort of acromegaly patients who have been previously demonstrated to have increased vertebral fracture risk, and compared this cohort to control patients with osteopenia or normal BMD. The results of this study are described in **Chapter 8**. All patients with well-controlled acromegaly attended the outpatient clinic of the Department of Endocrinology of the LUMC and are part of a well-characterized, hormonally and metabolically well-controlled cohort of patients. We found significantly lower BMSi values in patients with well-controlled acromegaly compared to control patients with similar BMD values. BMSi values were similarly low in patients with acromegaly, with or without vertebral fractures. These data suggest that tissue-level properties of bone are altered in patients with acromegaly and may contribute to the increased bone fragility observed in these patients.

Over the last few years, data from our group and that of other research groups have been accumulating on the value of impact microindentation in the assessment of bone fragility. Although experience with the use of this technique has significantly increased, a number of clinically relevant questions remain as yet to be answered, such as the identification of inadequately performed measurements, the approach of the sometimes large variance of consecutive measurements, the choice of the preferred site of investigation (currently the non-dominant leg although there are no data to support this recommendation) and whether IMI could be used in patients with

localized pathology of the tibia, which is currently an exclusion criterion. A number of these relevant questions are addressed in the study described in **Chapter 9**, that used IMI to investigate tissue-level properties of cortical bone in both legs in patients with Paget's disease of bone at one tibia compared to bilateral measurements in patients without tibia pathology. In patients without tibia pathology, we observed a minimal variance of sequentially obtained adequate measurements. There was also no difference in mean BMSi values between the dominant and non-dominant leg. Interestingly, in patients with Paget's, mean BMSi of the affected tibia was lower compared to that of the healthy tibia, albeit not reaching significance (74.7 ± 1.7 and 78.7 ± 1.3 ; $p = 0.120$). This finding was rather unexpected since Paget's disease is associated with significant alterations in the microarchitecture of bone and with an increased risk of fracture at the site of the lesion. However, these intriguing results might be due to the limited sample size of our study. There was a significantly greater variability in sequentially obtained measurements in the Pagetic tibia compared to measurements obtained in the non-Pagetic tibia. These data suggest that the variability of measurements may provide additional relevant information on tissue-level properties of cortical bone in addition to that provided strictly by BMSi values.

FUTURE PERSPECTIVES

There is mounting evidence that impact microindentation is a promising technique which enables the measurement of aspects of bone fragility not solely captured by BMD values. It has been shown that IMI can be used to differentiate patient groups with conditions known to negatively affect bone health such as type 2 diabetes mellitus, use of glucocorticoids and presence of fragility fractures from control patients. Although knowledge and experience with the use of IMI is steadily increasing, a number of aspects of the interpretation of data generated by this technique such as the relationship between BMSi and age and its relationship with BMD values, or the relationship of BMSi with traditional measurements of material properties of bone such as toughness remain unclear. Large clinical studies are required to unravel these issues. In addition, the observation of variable BMSi values in controls groups from different studies which range between 70 to 85 is very intriguing. This is clearly reflected in a study that demonstrated significantly lower BMSi values in Norwegian compared with Spanish women. These observations suggest that reference values should be established for different geographical groups, in addition to different age groups, before BMSi could be adequately interpreted on an individual patient basis.



Chapter 11

Nederlandse Samenvatting

INTRODUCTIE

Een groot deel van de bevolking loop tenminste eenmaal in hun leven een fractuur op. Patiënten met een hogere leeftijd hebben een grotere kans op het ontwikkelen van fracturen. Het krijgen van een fractuur zonder adequaat trauma is een teken dat er mogelijk sprake is van onderliggende osteoporose. Fracturen behoren tot de klinisch meest relevante uitkomsten van osteoporose en zijn gerelateerd met een toegenomen morbiditeit en mortaliteit en met hogere kosten voor de gezondheidszorg. Verder is de aanwezigheid van een fractuur positief voorspellend voor het krijgen van eventuele toekomstige fracturen.

Zogenaamde “fractuurklinieken” zijn multidisciplinaire samenwerkingsverbanden met het doel om patiënten te identificeren die recent een fractuur hebben gehad en om deze patiënten vervolgens, indien nodig, te behandelen met anti-osteoporose therapie. De Wereldgezondheidsorganisatie (WHO) heeft in 2013 wereldwijd de campagne “Capture the Fracture” gelanceerd om de ontwikkeling en toepassing van deze klinieken te stimuleren met als einddoel adequate secundaire preventie van fracturen bij patiënten die recent een fractuur hebben doorgemaakt. Fractuurklinieken leiden op een kosteneffectieve wijze tot een reductie in mortaliteit en in het aantal toekomstige fracturen.

Dit proefschrift is voor een groot deel gebaseerd op gegevens van patiënten vanuit de fractuurkliniek van het Leids Universitair Medisch Centrum (LUMC). Hoewel het LUMC een tertiair doorverwijzingscentrum is, verzorgt de fractuurkliniek van het LUMC de acute opvang voor alle fractuurpatiënten in de regio Leiden. De fractuurpatiënten die geschikt zijn voor doorverwijzing naar de fractuurkliniek worden uit de spreekuren van de polikliniek traumatologie en orthopedische chirurgie geïdentificeerd door een doktersassistente osteoporose. Deze aanpak is zeer succesvol gebleken en leidde tot een percentage doorverwijzingen van meer dan 50% gedurende de eerste twee jaar en steeg naar 95% in 2016.

DEEL 1: DE FRACTUURKLINIEK

Hoofdstuk 2, 3 en 4 beschrijven een grote groep patiënten van 50 jaar en ouder die tussen juni 2012 en juni 2014 vanwege een recente fractuur zijn doorverwezen naar de fractuurkliniek van het LUMC. Het screeningsprogramma dat wordt gebruikt in de fractuurkliniek bestaat uit een aantal onderdelen, ten eerste worden patiënten geanalyseerd op de aanwezigheid van osteoporose door middel van een botdichtheidsmeting met een DXA-scanner. Ten tweede wordt de aanwezigheid van onderliggende secundaire oorzaken voor osteoporose geëvalueerd door middel van

het verzamelen van klinische gegevens zoals leeftijd, geslacht, lengte en gewicht, fractuurvoorgeschiedenis en de aanwezigheid van risicofactoren voor osteoporose zoals gebruikt in het FRAX algoritme en door middel van laboratoriumonderzoek. Als laatste wordt er gezocht naar de aanwezigheid van wervelinzakkingen door middel van de VFA-functie op het DXA-apparaat en door middel van röntgenfoto's van de thoracale en de lumbale wervelkolom.

In **hoofdstuk 2** wordt de prevalentie van onderliggende secundaire oorzaken voor osteoporose beschreven in bovenstaand beschreven groep van 686 patiënten uit de fractuurkliniek van het LUMC. Wij zagen een groot aantal onderliggende oorzaken voor osteoporose bij patiënten met een recente fractuur ongeacht de botdichtheid; 47% van de patiënten met een normale botdichtheid, 57% van de patiënten met osteopenie en 62% van de patiënten met osteoporose hadden een of meer onderliggende oorzaken voor osteoporose. Verder zagen wij een relatie tussen het aantal onderliggende oorzaken per patiënt en de botdichtheid. Klinisch relevant is dat meer dan de helft van het aantal onderliggende oorzaken reversibel is, mogelijk leidt het wegnemen of het opheffen hiervan tot een vermindering van het risico op nieuwe fracturen. Vanwege de hoge prevalentie onderliggende oorzaken voor osteoporose bij patiënten met een recente fractuur onafhankelijk van de botdichtheid, adviseren wij dat er niet alleen bij de patiënten met osteoporose naar de aanwezigheid van deze oorzaken moet worden gezocht, maar bij alle fractuurpatiënten.

De resultaten van onze studie naar de waarde van VFA-onderzoek voor het identificeren van wervelinzakkingen bij patiënten boven de 50 jaar met een recente fractuur worden beschreven in **hoofdstuk 3**. Vertebral Fracture Assessment (VFA) is een onderzoek dat in dezelfde meetsessie als de botdichtheidsmeting kan worden uitgevoerd met het DXA-apparaat. Met een zeer lage hoeveelheid straling wordt laterale beeldvorming van de thoracale en laterale wervelkolom gemaakt, waarop vervolgens kan worden beoordeeld of er sprake is van wervelinzakkingen. De sensitiviteit van VFA voor het identificeren van een patiënten met een wervelinzakking graad 2 of hoger was 0.77 en de specificiteit was 0.80. Dit houdt in dat het VFA-onderzoek 23% van alle patiënten met een wervelinzakking graad 2 of hoger heeft gemist. Deze uitkomsten wijken sterk af van onderzoeksuitkomsten uit de literatuur en om deze reden is er naast bovenstaande analyse ook een systematische review en een meta-analyse uitgevoerd naar de waarde van VFA-onderzoek, deze staan eveneens beschreven in **hoofdstuk 3**. Onze systematische review leidde tot 16 verschillende studies waarin totaal 3238 patiënten waren geïncludeerd. De gerapporteerde sensitiviteit en specificiteit van de studies waren echter zeer verschillend (respectievelijk 47–99% en 74–100%). Mogelijk is dit veroorzaakt door de variabiliteit in de studie opzet en in de

inclusie criteria van patiënten. De meta-analyse toonde een sensitiviteit van 0.82 (95% BI, 0.75–0.87) en een specificiteit van 0.99 (95% BI, 0.98–1.00) voor het identificeren van wervelinzakkingen graad 1 of hoger door VFA-onderzoek. Samenvattend waren de resultaten van zowel de systematische review als de meta-analyse discrepant hoog in vergelijking met de resultaten van onze eigen studie. De verschillen kunnen worden verklaard door verschillen in studie opzet en in het gebruik van variabele software en hardware. Vanwege deze bevindingen adviseren wij om de uitkomsten van de VFA-methode in ieder ziekenhuis te controleren voordat deze methode in plaats van röntgenfoto's van de laterale wervelkolom wordt gebruikt om de aanwezigheid van wervelinzakkingen te evalueren.

De mate van formatie en resorptie van botweefsel kan worden gemeten met behulp van botturnovermarkers. Botturnovermarkers kunnen gemakkelijk worden gemeten in serum van de patiënt en zijn potentieel geschikt voor het vervolgen van het therapie-effect van anti-osteoporose medicatie. In **hoofdstuk 4** wordt het effect van anti-osteoporose medicatie op de botturnovermarkers P1NP en Ctx beschreven bij patiënten met een recente fractuur, daarnaast wordt de relatie tussen deze botturnovermarkers en de botdichtheid beschreven. Er is een omgekeerde relatie tussen het verschil in P1NP gemeten na 3 en 12 maanden therapie en het verschil in botdichtheid van de lumbale wervelkolom na 12 maanden therapie. Na correctie voor tijd tussen het oplopen van de fractuur en starten van therapie was alleen de relatie tussen de botturnovermarkers en de botdichtheid van de lumbale wervelkolom 12 maanden na het starten van therapie statistisch significant. Er is ook een omgekeerde relatie tussen het verschil in Ctx gemeten na 12 maanden therapie en het verschil in botdichtheid van de lumbale wervelkolom na 12 maanden therapie nadat er is gecorrigeerd voor tijd tussen het oplopen van de fractuur en starten van therapie. Verder wordt er een relatie gezien tussen de P1NP waarde voor het starten van anti-osteoporose medicatie en het verschil in botdichtheid van de lumbale wervelkolom gemeten na 12 maanden. Deze studie toont aan dat het vroege effect op botturnovermarkers na het starten van antiresorptieve therapie voorspellend is voor het effect op de botdichtheid door deze therapie. Of botturnovermarkers voorspellend zijn voor het risico op fracturen moet worden aangetoond in grote longitudinale studies.

DEEL 2: IMPACT MICROINDENTATIE

In **hoofdstuk 6 tot en met 9** worden de resultaten van de studies naar impact microindentatie (IMI) beschreven. IMI is een nieuwe methode voor het onderzoeken van materiaal eigenschappen van bot bij de mens en wordt uitgevoerd met de Osteoprobe®.

De methode is gebaseerd op de hypothese dat een fractuur het gevolg is van de scheiding van gemineraliseerde collageenvezels, waardoor microscheurtjes in het bot ontstaan. De metingen worden bij mensen met slechts plaatselijke anesthesie op de anterieure zijde van de tibia uitgevoerd. Voor de meting wordt er een meetnaaldje in de Osteoprobe geplaatst en de naald wordt met een kracht van 40 Newton tegen het botoppervlakte gedreven. Deze handeling heeft microscheurtjes en micro-indentaties tot gevolg en wordt een aantal keer herhaald. De grootte van de micro-indentatie wordt de "Indentation Distance Increase" (IDI) genoemd. Hierna wordt dezelfde handeling herhaald op fantoommateriaal gemaakt van polymethylmethacrylate. Met de metingen op het bot en op de fantoom kan de "Bone Material Strength index" (BMSi) worden berekend met de formule $100 \times (\text{harmonisch gemiddelde van de IDI op de fantoom} / \text{gemiddelde van de IDI op het bot})$. Dat wil zeggen dat de BMSi waarde 100 zal zijn als het bot precies zo sterk is als de fantoom. Indien het bot minder weerstand heeft tegen de indentaties dan zal de BMSi lager zijn. In **hoofdstuk 5** wordt er een uitgebreid overzicht gegeven over de ontwikkeling van IMI en over de klinische studies die tot op heden zijn gepubliceerd.

In **hoofdstuk 6** wordt een studie beschreven waarin de BMSi waarde is gemeten van 90 patiënten met een lage botmassa (osteopenie en osteoporose) en zowel met als zonder fracturen. Patiënten met een fractuur hebben gemiddeld een lagere BMSi waarde dan patiënten zonder een fractuur. Verder zagen wij een sterke omgekeerde relatie tussen BMSi en de leeftijd van de patiënt. Deze gegevens impliceren dat de materiaal eigenschappen van bot bij mensen met een fractuur afwijkend zijn en dat BMSi metingen parameters van botsterkte omvatten die niet worden omvat door een botdichtheidsmeting.

Nadat de studie beschreven in **hoofdstuk 6** was afgerond en gepubliceerd, werden er twee studies gepubliceerd waarin de relatie tussen BMSi en wervelfracturen werd onderzocht. De resultaten van deze twee studies waren echter tegenstrijdig. Om meer inzicht te krijgen of BMSi inderdaad inzicht kan geven in materiaaleigenschappen van het gehele skelet in plaats van alleen corticaal bot, onderzochten wij de relatie tussen BMSi en verscheidene typen fracturen, waaronder wervelfracturen, waarvan de resultaten staan beschreven in **hoofdstuk 7**. Er werden in totaal 132 patiënten geïncludeerd in deze studie, waaronder 101 patiënten met een fractuur. De fractuurpatiënten konden worden verdeeld in 14 patiënten met een wervelfractuur, 53 met een niet-wervel fractuur en 34 met zowel een wervelfractuur als een ander fractuurtype. De BMSi van de patiënten met de verschillende fractuurtypen was onderling niet verschillend, maar was wel lager dan de BMSi van patiënten zonder een fractuur. Deze studie laat

daarom zien dat BMSi een weergave is van botsterkte in het gehele skelet en niet alleen van botsterkte van het corticale bot.

Met het doel te onderzoeken of de IMI techniek in staat is patiënten met een hoog risico op wervelfracturen te identificeren hebben wij een studie uitgevoerd waarin BMSi waarden bij 48 patiënten met acromegalie werden gemeten. De resultaten van dit onderzoek staan beschreven in **hoofdstuk 8**. Acromegalie is een zeldzame endocriene ziekte waarbij er een overproductie van groeihormoon bestaat met een scala van gevolgen, waaronder een hoge prevalentie wervelfracturen. De acromegalie patiënten werden vergeleken met 44 controle patiënten met vergelijkbare BMI en botdichtheid van de wervelkolom en het collum femoris. Er werd een gemiddeld lagere BMSi waarde gezien bij patiënten met acromegalie dan bij de controle patiënten. In de vergelijking tussen acromegalie patiënten met en zonder wervelfracturen werd er geen verschil in BMSi gezien. De resultaten van deze studie impliceren dat de materiaal eigenschappen van het bot bij patiënten met acromegalie afwijkend zijn wat mogelijk bijdraagt aan het hoge risico op wervelfracturen.

In de periode waarin de studies werden uitgevoerd die staan beschreven in dit proefschrift, werd eveneens door andere onderzoeksgroepen onderzoek uitgevoerd met de IMI methode. Deze studies hebben allen bijgedragen aan een ruimere ervaring met de methode maar leidden ook tot een aantal vragen die reeds werden benoemd in verschillende review artikelen. Het gaat om de volgende aandachtspunten: de identificatie van foutmetingen, de variabiliteit van afzonderlijke metingen op het botoppervlakte en de voorkeurslocatie van de metingen op het bot (oftewel het dominante of het niet-dominante been). Deze vraagstukken worden allen onderzocht in de studie beschreven in **hoofdstuk 9**, waarin wij BMSi metingen hebben uitgevoerd op beide tibiae bij patiënten met de ziekte van Paget gelokaliseerd in de tibia en bij patiënten zonder afwijkingen in de tibia. Er werd een minimale variantie van de uitgevoerde afzonderlijke botmetingen gezien bij de meerderheid van de patiënten zonder pathologie aan de tibia. Een grotere variantie werd ook gezien, maar dit leidde bij geen van de patiënten tot een significant verschil in de gemiddelde BMSi waarde van de contralaterale tibia. Er was geen significant verschil in BMSi tussen de dominante en niet-dominante tibia in deze patiënten. Bij de patiënten met de ziekte van Paget was de BMSi waarde van de tibia waarin de Paget laesie was gelokaliseerd lager dan de BMSi waarde van de contralaterale tibia zonder afwijkingen, dit verschil was echter niet significant lager. Laatstgenoemd resultaat bij de patiënten met de ziekte van Paget was onverwachts aangezien de ziekte gepaard gaat met forse afwijkingen in microarchitectuur van het aangedane bot en met een verhoogd risico op fracturen. Ondanks het ontbreken van een significant verschil in BMSi, was er wel een grotere

variabiliteit van sequentiële metingen bij de aangedane tibiae dan bij de tibiae zonder afwijkingen. Deze studie laat zien dat de variabiliteit van de metingen als een nieuwe parameter voor materiaal eigenschappen van bot kan worden gebruikt en suggereert dat metingen als foutief moeten worden beoordeeld door een ervaren *operator* in plaats van door een geautomatiseerd algoritme waarin pre-gedefinieerde afkapwaarden worden gehanteerd.

TOEKOMSTPERPECTIEF

Impact microindentatie is een veelbelovende techniek die parameters van botsterkte weergeeft die niet worden weergegeven door een botdichtheidsmeting. Eerdere studies hebben aangetoond dat IMI kan differentiëren tussen patiëntgroepen met een aandoening die gepaard gaat met een verhoogd fractuurrisico zoals diabetes mellitus type 2, het gebruik van glucocorticoïden en de aanwezigheid van een fractuur, en controle patiënten. Ondanks dat de kennis en de ervaring van deze techniek toeneemt bestaan er een aantal vragen en discussiepunten die nog onduidelijk zijn, zoals de relatie tussen BMSi en leeftijd of de botdichtheid van de patient, en de relatie tussen BMSi en klassieke mechanische parameters. Het is noodzakelijk dat deze onduidelijkheden inzichtelijk worden gemaakt. Het is opmerkelijk dat de gemiddelde BMSi waarden van de controle patienten in de gepubliceerde studies erg variëren, namelijk van 70 tot 85. Een studie toonde eerder een significant verschil in BMSi aan tussen vrouwen uit Noorwegen en uit Spanje. Deze resultaten suggereren dat het nodig zal zijn om referentiewaarden te implementeren waarin wordt gecorrigeerd voor karakteristieken zoals leeftijd en geografische achtergrond voordat uitkomstwaarden kunnen worden vergeleken en op een individuele basis kunnen worden gebruikt.

LIST OF ABBREVIATIONS

AFF	Atypical femoral fractures
BMD	Bone Mineral Density
BMI	Body Mass Index
BMSi	Bone Material Strength index
BMSu	Unnormalized Bone Material Strength
BPiv	Intravenous bisphosphonates
BPor	Oral bisphosphonates
BTM	Bone turnover marker
CAG	Chronic atrophic gastritis
CA/D	Calcium/vitamin D supplementation
CKD	Chronic kidney disease
CMI	Cyclic reference point microindentation
CTX	C-terminal crosslinked telopeptide of type I collagen
CV	Coefficient of variation
DALY	Disability Adjusted Life Years
Dmab	Denosumab
DXA	Dual Energy X-ray Absorptiometry
eGFR	estimated glomerular filtration rate
EM	early menopause
ER	Emergency Room
FLS	Fracture Liaison Service
FN	Femoral neck
FU	Follow-up
GD1	Type 1 Gaucher disease
GH	Growth hormone
GP	General practitioner
HG	Hypogonadism
HIV	Human Immunodeficiency Virus
HT	Hyperthyroidism
HRpQCT	High Resolution peripheral Quantitative Computed Tomography
IDI	Indentation Distance Increase
IGF-1	Insulin-like Growth Factor-1
IMI	Impact microindentation
IU	International Unit
LS	Lumbar spine
LUMC	Leiden University Medical Center
MDRD	Modification of diet in renal disease

MGUS	Monoclonal Gammopathy of Undetermined Significance
NHANES	National Health and Nutrition Survey
NH/NV	non-hip/non-vertebral
OR	Odds ratio
PMMA	Polymethylmetacrylate
PTH	Parathyroid hormone
P1NP	Procollagen 1 N-terminal extension peptide
RA	Rheumatoid arthritis
RPI	Reference Point Indentation
RSN	Risedronate
SD	Standard deviation
SEM	Standard error of the mean
SERMs	Selective estrogen receptor modulators
TBS	Trabecular Bone Score
TPTD	Teriparatide
T2DM	Type 2 Diabetes Mellitus
VFA	Vertebral Fracture Assessment
VF+/Fx+	Vertebral and non-vertebral fracture
VF+/Fx-	Only vertebral fracture
VF-/Fx+	Only non-vertebral fracture
WHO	World Health Organization
1° HPT	Primary hyperparathyroidism
2° HPT	Secondary hyperparathyroidism
95% CI	95% confidence interval

LIST OF PUBLICATIONS

1. Ray A , Huisman MV, Tamsma JT; Research and Writing-group, van Asten J, Bingen BO, Broeders EA, Hoogeveen ES, van Hout F, Kwee VA, Laman B, **Malgo F**, Mohammadi M, Nijenhuis M, Rijke M, van Tellingen MM, Tromp M, Tummers Q, de Vries L. The role of inflammation on atherosclerosis, intermediate and clinical cardiovascular endpoint in type 2 diabetes mellitus. *Eur J Intern Med* 2009 May;20(3):253-60.
2. Appelman-Dijkstra NM, **Malgo F**, Neelis KJ, Coremans I, Biermasz NR, Pereira AM. Pituitary dysfunction in adult patients after cranial irradiation for head and nasopharyngeal tumours. *Radiother Oncol.* 2014 Oct;113(1):102-7.
3. **Malgo F**, Hamdy NA, Papapoulos SE, Appelman-Dijkstra NM. Bone material strength as measured by microindentation in vivo is decreased in patients with fragility fractures independently of bone mineral density. *J Clin Endocrinol Metab.* 2015 May;100(5):2039-45.
4. **Malgo F**, Appelman-Dijkstra NM, Termaat MF, van der Heide HJ, Schipper IB, Rabelink TJ, Hamdy NA. High prevalence of secondary factors for bone fragility in patients with a recent fracture independently of BMD. *Arch Osteoporos.* 2016;11:12.
5. **Malgo F**, Hamdy NA, Rabelink TJ, Kroon HM, Claessen KM, Pereira AM, Biermasz NR, Appelman-Dijkstra NM. Bone material strength index as measured with impact microindentation is altered in patients with acromegaly. *Eur J Endocrinol.* 2017 Mar;176(3):339-347.
6. **Malgo F**, Hamdy NAT, Papapoulos SE, Appelman-Dijkstra NM. Bone material strength index as measured with impact microindentation is low in patients with fracture irrespective of fracture site. *Osteoporosis International.* 2017 Aug;28(8):2433-2437.
7. **Malgo F**, Hamdy NAT, Ticheler CHJM, Smit F, Kroon HM, Rabelink TJ, Dekker OM, Appelman-Dijkstra NM. Value and potential limitations of Vertebral Fracture Assessment (VFA) compared to conventional spine radiography: Experience from a Fracture Liaison Service (FLS) and a meta-analysis. *Osteoporos Int.* 2017 Oct;28(10):2955-2965.
8. **Malgo F**, Hamdy NAT, Papapoulos SE, Appelman-Dijkstra NM. Impact microindentation: consistency of serial measurements and alterations in patients with Paget's

disease of the tibia. *Accepted for publication in Journal of Bone and Mineral Research*

9. **Malgo F**, Appelman-Dijkstra NM, Wolberink IM, Beckers PH, Hamdy NAT. Biochemical markers of bone turnover in the management of patients receiving osteoporosis treatment after a recent fracture. *Submitted*

CURRICULUM VITAE

Frank Malgo werd geboren op 13 oktober 1987 te Vlissingen. Hij behaalde in 2005 zijn VWO diploma aan de Stichting Scholengemeenschap Scheldemonde te Vlissingen, waarna hij in hetzelfde jaar startte met de studie Geneeskunde aan de Universiteit Leiden. Na het behalen van het arts examen in 2011, werden de eerste ervaringen in de patiëntenzorg opgedaan als ANIOS bij de afdeling Interne Geneeskunde van het Alrijne Ziekenhuis te Leiderdorp. Vanwege zijn interesse in wetenschappelijk onderzoek startte hij ruim een jaar later aan zijn promotietraject “Diagnostic strategies in patients with high fracture risk” bij de afdeling Endocrinologie van het Leids Universitair Medisch Centrum onder supervisie van prof. dr. A.J. Rabelink, dr. N.M. Appelman-Dijkstra en dr. N.A.T. Hamdy. Inherent aan dit onderzoekstraject werden deze werkzaamheden gecombineerd met patiëntenzorg bij het recent opgestarte Zorgpad Osteoporose. Vanaf oktober 2016 heeft hij als ANIOS gewerkt bij de afdeling Klinische Geriatrie van het Diaconessenhuis te Zeist en Utrecht. Per december 2017 zal Frank starten met de opleiding tot klinisch geriater (opleider: dr. F.U.S. Mattace Raso, Erasmus MC), hij start met de vooropleiding bij de afdeling Interne Geneeskunde van het Spaarne Gasthuis te Haarlem en Hoofddorp (opleider dr. W. de Ronde).

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