

Bone geometry, bone mineral density, and micro-architecture in patients with myelofibrosis: a cross-sectional study using DXA, HR-pQCT, and bone turnover markers

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Abstract Primary myelofibrosis (MF) is a severe chronic myeloproliferative neoplasm, progressing towards a terminal stage with insufficient haematopoiesis and osteosclerotic manifestations. Whilst densitometry studies have showed MF patients to have elevated bone mineral density, data on bone geometry and micro-structure assessed with non-invasive methods are lacking. We measured areal bone mineral density (aBMD) using dual-energy X-ray absorptiometry (DXA). Bone geometry, volumetric BMD, and micro-architecture were measured using high-resolution peripheral quantitative computed tomography (HR-pQCT). We compared the structural parameters of bones by comparing 18 patients with MF and healthy controls matched for age, sex, and height. Blood was analysed for biochemical markers of bone turnover in patients with MF. There were no significant differences in measurements of bone geometry, volumetric bone mineral density, and

micro-structure between MF patients and matched controls. Estimated bone stiffness and bone strength were similar between MF patients and controls. The level of pro-collagen type 1 N-terminal pro-peptide (P1NP) was significantly increased in MF, which may indicate extensive collagen synthesis, one of the major diagnostic criteria in MF. We conclude that bone mineral density, geometry, and micro-architecture in this cohort of MF patients are comparable with those in healthy individuals.

Keywords Myelofibrosis · Bone structure · Bone geometry · Bone mineral density · Osteosclerosis · Bone turnover

Introduction

Primary myelofibrosis (MF) is one of the classical Philadelphia chromosome-negative chronic myeloproliferative neoplasms (CMPN) also including essential thrombocythemia (ET), polycythaemia vera (PV), and others [1]. With time a progression across the biological CMPN continuum from ET and PV to secondary myelofibrosis may evolve. Some patients undergo the full spectrum with the initial proliferative stage that gradually diminishes due to the increasing fibrosis eventually resulting in a burn-out terminal stage with bone marrow failure [2, 3]. Accordingly, manifestations of extra-medullar hematopoiesis arise, spleen size increases, and constitutional symptoms may compromise quality of life [4, 5]. As the name myelofibrosis refers, increasing reticulin or collagen fibres are discriminating features of the bone marrow [2]. Along with this, osteosclerotic changes appear in the bone tissue as a result of the growing increase and thickening of bone trabeculae thickness and focal osteosclerotic changes

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with osteoid seams and appositional new bone formation in budding plaques [6]. Bone density assessed using dual-energy X-ray absorptiometry (DXA) in MF patients has been found to be higher than the background population [7, 8]. Histomorphometric measurements in a cohort of 75 CMPN patients have showed that MF patients have significantly more bone tissue compared with other CMPN type patients, and a positive correlation between the amount of bone and the degree of fibrosis [9]. Radiological abnormalities visualized by conventional X-ray such as endosteal osteosclerosis have been reported, most common in the axial skeleton and the metaphyseal ends of the long bones [10].

Recently, we were the first to show that patients with the classical CMPNs have a higher incidence rate and risk of fractures than age- and sex-matched controls from the general population [11]. The CMPN patients in our previous study were defined by their WHO International Classification of Diseases ICD-8 or ICD-10 diagnosis code. Previously, a specific diagnosis code for MF was not comprised in the ICD; therefore, this CMPN subtype was not included. Also, due to the registry-based nature of the data on disease or treatment specific, details were not available for the included patients [11].

Osteoporosis is characterized by low bone mass and micro-architectural deterioration [12]. It is well documented that areal bone mineral density (aBMD) measured by dual-energy X-ray absorptiometry (DXA) is an important factor in predicting osteoporotic fractures [13]. However, aBMD is limited by assessing only the bone mass and does not capture all fractures [14, 15]. Today it is possible to assess micro-architecture by high-resolution peripheral quantitative computed tomography (HR-pQCT) with distal measurements of volumetric BMD and compartmental architecture at the radius and tibia with a voxel size of 82 μm [16].

Further, with measurements of biochemical bone turnover markers (BTM), complementary information of bone metabolism can be achieved, with information of bone formation as well as resorption, reflecting the global bone metabolism, in contrast to imaging modalities measuring defined focal areas [17].

The absolute fracture risk in MF is unknown, but since MPNs are perceived as different phenotypic presentations of the same disease, the aim of this clinical study is to assess bone geometry, bone mineral density, and micro-architecture in patients with myelofibrosis and indirectly assess fracture risk [18]. We report results from bone strength and structure investigations amongst 18 patients with MF including dual-energy X-ray absorptiometry (DXA), and high-resolution peripheral quantitative computed tomography (HR-pQCT). The latter method has not been adopted amongst patients with MF previously. We

also assess bone turnover by measurements of biochemical markers in peripheral blood.

Methods

We conducted a cross-sectional study including all patients with either a diagnosis of primary myelofibrosis or secondary myelofibrosis following PV or ET according to WHO 2008-criteria from the department of Haematology, Odense University Hospital, Denmark covering the period 2012–2014.

In Denmark, the entire population has a unique and permanent 10-digit registry number (the CPR number), and each contact with the government-based healthcare system is registered by this number in both patient electronic files as well as the Danish National Registry of patients (DNRP) together with their diagnostic code [19]. This enables a linkage between specific diagnostic codes and individuals, and thereby identification of well-defined cohort of patients. The earlier diagnostic coding for MF was unspecific in the nomenclature “morbus myeloproliferativa chronicus”, and leads to mis-classifications, but with the latest classification named “primary or secondary myelofibrosis” the diagnostic coding improved. At our clinic, a revision and update to the latest classification of all living MF patients have been performed, which allowed us to identify a complete group of patients diagnosed with myelofibrosis at the department of Haematology, and by their CPR number link to their medical files. The files of all eligible patients were reviewed to ensure all patients met the diagnostic WHO 2008 MF criteria. After review of files MF patients were disregarded if they met any of the following exclusion criteria: Dementia, terminal illness, age above 80 years (since matching controls were all under 80 years), pregnancy, Paget’s disease, multiple myeloma, hyperparathyroidism, osteomalacia, liver or kidney disease, medical treatment with anti-oestrogen drugs, anti-osteoporotic drugs or use of glucocorticoids more than 5 mg/day for 3 months or more. In all 27 MF eligible patients were identified. Of these, 18 were included in the study. Seven patients were excluded by pre-defined exclusion criteria (one dementia, five aged 80 years or more, and one terminally ill) and two declined participation.

Age-matched controls were identified in a database with normative and descriptive data of men and woman from a Caucasian population ($N = 499$) recruited from the general population of the municipality of Odense [20]. The population in this database has participated in previous studies to generate descriptions of bone structure and strength measures amongst the Danish population [20]. Each MF patient was matched with respect to age (± 2 years), sex, and height (± 5 cm).

Ethics

All participants were informed both in person by SF and written, and informed consent was obtained according to the National GCP Guidelines. The study was approved by Ethics Committee of Southern Denmark S-20110110 as well as the Danish data protecting agency nr. 2008-58-0035.

Diagnosis validation

All patients had previously at least one bone marrow examination done to meet the diagnostic criteria, and new bone marrow biopsies were not requested for this study. For the purpose of this study, all previous bone marrow biopsies were re-examined by one of the authors (CS) who is an experienced haematopathologist. This was done to confirm the histopathological MF diagnosis, to validate the classification of fibrosis grade, as well as the presence vs. the absence of osteosclerotic bone marrow changes. Grades of bone marrow fibrosis were after pathology review subsequently categorized in two groups: MF grade 1–2 and MF grade 3. Janus kinase 2 mutational status (JAK2) was known in all patients [21].

Dynamic International Prognostic Scoring System (DIPPS Plus score) that incorporates age >65, haemoglobin >10 g/L, leucocytes >25 × 10⁹ g/L, circulating blasts >1 % and constitutional symptoms besides karyotype, platelet count, and transfusion status was assessed at all patients at the time of the inclusion in the study as a prognostic tool, to assess the severity of disease of the patients [22, 23]. Patients were stratified into four groups; low risk, intermediate 1, intermediate 2 and high risk.

Dual-energy X-ray absorptiometry (DXA)

Areal BMD (aBMD) were measured of the lumbar spine (L1–L4) and the non-dominant total hip region using DXA (Hologic Discovery, Waltham, MA, USA). Coefficient of variation (CV) for measurements of total hip and spine in our clinic was 1.5 % at each site.

High-resolution peripheral quantitative computed tomography (HR-pQCT)

Bone geometry, vBMD, micro-architecture, and bone strength were assessed using an HR-pQCT system (Xtreme CT, Scanco Medical, AG, Brüttisellen, Switzerland) at the non-dominant distal radius and distal tibia (the opposite limb in the presence of a previous fracture). The manufacturer default protocol for in vivo imaging that has been described previously [16] was applied, providing a 9.02-mm axial 3D representation at each anatomical site. Each measurement was initiated at 9.5 and 22.5 mm from

the mid-endplate at the radius and tibia respectively and included 110 parallel slices in the axial direction providing images with an isotropic image voxel size of 82 μm. The following outcome variables were included in our analysis using the manufacturer's standard software: bone geometrical measures including total, cortical and trabecular areas (mm²), volumetric BMD (vBMD) (mg hydroxyapatite/cm³) for the entire (Total vBMD), trabecular (Tb vBMD) and cortical regions (Ct vBMD), and trabecular micro-architectural parameters including trabecular bone volume to tissue volume fraction (Tb BV/TV), trabecular number (Tb.N, mm⁻¹), thickness (Tb.Th, mm), separation (Tb.Sp, mm) and trabecular network inhomogeneity (standard deviation of 1/Tb.N, mm). In addition, periosteal and endosteal surfaces of the cortex were extracted using a specific cortical evaluation software allowing 3D measurement of cortical thickness (periosteal–endosteal distance, Ct.Th, mm) and cortical porosity (Ct.Po, %) as previously described [24, 25]. Finally, total bone stiffness (N/mm) and estimated failure load (kN) were assessed as indices of bone strength using a micro-FEA solver provided by the manufacturer (Finite Element analysis Software v1.15, Scanco Medical, Switzerland) [26, 27].

Image quality was assessed by one of the authors (VS) using the 5-step scale with 1 being the best and 5 being the worst [28] and images more than grade 3 were excluded from the analysis. A maximum of three scans per anatomical site was allowed to obtain images of optimal quality. In our unit, CVs for geometry, density, and micro-architecture parameters ranged from 0.4 to 7.2 % [29].

Biochemical analysis

Blood samples were obtained in a fasting state from the MF patients. A biochemical screen for conditions or endocrinal abnormalities that could influence normal bone homeostasis was performed including haemoglobin, leucocytes, thrombocytes, lactate dehydrogenase (LDH), thyroid-stimulating hormone (TSH), parathyroid hormone (PTH), ionized calcium (Ca²⁺), phosphate (P), 25-hydroxyvitamin D (25-OH-vitamin D), and alkaline phosphatase (BASP). These analyses were done at the department of Clinical Biochemistry, Odense University Hospital.

We collected blood samples for the purpose of measuring the biochemical bone markers: pro-collagen type 1 N-terminal pro-peptide (P1NP), osteocalcin (OC), C-terminal cross-linking telopeptide of type 1 collagen (CTX), and bone-specific alkaline phosphatase (bALP). Blood samples were pipetted into small tubes and frozen to –80 °C and stored until time for analyses.

P1NP was analysed by manual enzyme-linked immunosorbent assay (ELISA) at Institute of Cancer and Inflammation, University of Southern Denmark [30]. OC was

analysed by automated electro-chemiluminescence immunoassay (ECLIA) at Unilabs laboratories, Copenhagen; bALP was analysed by manual ELISA (MicroVue™ BAP) and CTX by automated Roche modular, β -cross Laps/serum immunoassay, both at department of Clinical Immunology and Biochemistry, Vejle Hospital). Variation of analysis and intra-individual biologic variations are specified from the individual labs, respectively, for P1NP: 5 and 5 %, for CTX 5 and 18 %, for bALP 7 and 6 % and not given for OC.

P1NP levels were compared with samples from department of Clinical Immunology, Odense University Hospital from 18 healthy blood donors (aged 50–70) who served as reference subjects; remaining BTM were compared with reference values directed from the respective laboratories.

Statistical analysis

Data are presented as medians with 25–75 % inter-quartile range (IQR). Wilcoxon non-parametric rank-sum test was used to compare results of imaging modalities between MF patients and healthy controls, as well as P1NP levels in MF patient and healthy blood donors. Due to the size of the data set, data were generally not normally distributed, and non-parametric methods were therefore used. STATA statistical software release 12.0 was used for the analyses [31]. Level of statistical significance was set at a two-sided P value <0.05.

Results

Patients

The MF patients included in the study had a median age of 68.2 years (IQR 63.8–71.3 years) and with an even distribution of males and females (Supplementary Table 1). Anthropometrics for MF patients and their respective control subjects are shown in Supplementary Table 1. MF patients and controls were comparable with regard to age, height, weight, and BMI. Patients were scanned 1341 (IQR 868–1877) days after diagnosis, ranging from 123 to 2608 days. Assessment with DIPPS plus categorized no patients as low risk, 4 patients as intermediate-1 (22.2 %), 11 patients as intermediate-2 (61.1 %), and 3 patients as high risk (16.7 %) Table 1.

Bone marrow evaluation

The diagnostic criteria as well as grading of myelofibrosis were confirmed in all patients after pathology review. JAK2 mutation was present in 12 patients. Nine patients

were classified as grade MF 1–2 and nine patients as grade MF-3 fibrosis in their bone marrow biopsy. Osteosclerosis including focal osteosclerosis was observed in three of the patients with MF grade 1–2, and eight of the patients with MF grade 3.

Comparison of areal bone mineral density (aBMD) by DXA

Results of the bone densitometry are shown in Fig. 1. As it can be seen aBMD in the hip was highly comparable between MF patients and controls, whilst measurements in the spine showed a possible increased aBMD of MF patients relative to controls, but did not reach statistical significance (Fig. 1).

Total spine aBMD was 1.01 g/cm² (IQR 0.87–1.15 g/cm²) in MF patients and 0.92 g/cm² (IQR 0.83–0.99 g/cm²) in healthy controls, $P = 0.08$. Total hip aBMD was 0.95 g/cm² (IQR 0.86–1.05 g/cm²) in patients compared to 0.89 g/cm² (IQR 0.79–1.03 g/cm²) in the controls, $P = 0.35$.

Table 1 Biochemical measurements [median (25–75 % IQR)] and level of bone formation markers (P1NP, bALP, and osteocalcin) and bone resorption marker (CTX) in 18 patients with myelofibrosis

	MF patients ($N = 18$)	Reference values
Biochemistry		
Haemoglobin/g/dL females	11.6 (10.8–13.0)	11.7–15.3
Haemoglobin/g/dL males	10.8 (10.1–12.0)	13.4–19.9
Leucocytes/ $10E^9/L$	6.6 (2.9–9.4)	3.5–8.8
Platelets/ $10E^9/L$	275 (223–343)	165–400
Lactate dehydrogenase/U/L	379 (212–457)	155–255
Parathyroid hormone/pmol/L	7.0 (4.1–8.9)	1.1–6.9
Thyroid stimulating hormone/ $10E^{-3}$ int U/L	2.3 (1.1–2.6)	0.3–4.0
25-Hydroxyvitamin D/nmol/L	59 (32–80)	50–160
Ionized calcium/mmol/L	1.22 (1.18–1.25)	1.19–1.29
Phosphate/mmol/L	1.4 (1.1–1.3)	0.78–1.58
Creatine/ μ mol/L	86 (63–98)	45–90
Alkaline phosphatase/U/L	101 (81–102)	35–105
Bone formation markers		
P1NP (mg/L)	97 (77–125)	56 (42–68)
bALP (U/L)	20 (12–23)	12–43
Osteocalcin (μ /L)	26 (20–31)	14–45
Bone resorption markers		
CTX (μ g/L)	0.6 (0.4–0.8)	>0.84

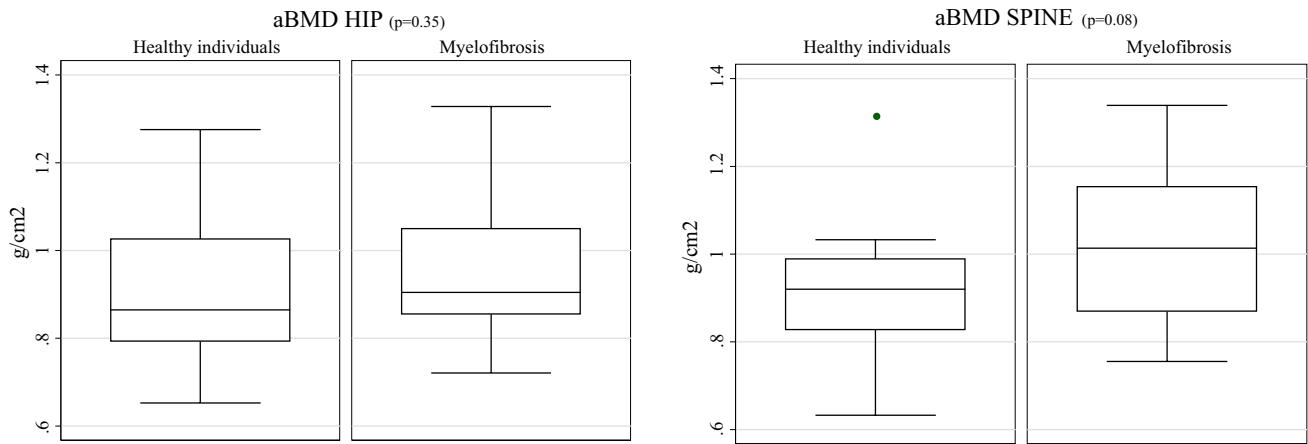


Fig. 1 Hip- and spine aBMD in MF patients compared with healthy individuals

Table 2 Bone geometry, volumetric bone mineral density, and micro-architecture by HR-pQCT; failure load by FEA in patients with myelofibrosis (MF) and healthy controls in distal radius

	MF patents (<i>N</i> = 16)	Healthy controls (<i>N</i> = 16)	<i>P</i> value
Bone geometry			
Total area (mm ²)	357.5 (300.4–438.4)	339.3 (257.3–422.9)	0.76
Cortical area (mm ²)	60.9 (47.2–75.9)	60.1 (43.6–79.6)	0.97
Trabecular area (mm ²)	288.3 (240.1–363.6)	269.1 (201.3–342.3)	0.51
Volumetric bone mineral density			
Total density (mg/cm ³)	298.5 (255.6–343.69)	304 (257.5–352.0)	0.85
Cortical density (mg/cm ³)	828.7 (787.4–880.8)	822.3 (772.5–873.5)	0.88
Trabecular density (mg/cm ³)	166.7 (140.3–198.2)	160.5 (121.8–203.0)	0.85
Micro-architecture			
Trabecular (BV/TV (%))	0.139 (0.17–0.165)	0.134 (0.102–0.169)	0.84
Trabecular number (mm ⁻¹)	2.03 (1.82–2.29)	1.90 (1.68–2.27)	0.40
Trabecular thickness (mm)	0.128 (0.066–0.075)	0.071 (0.060–0.081)	0.65
Trabecular spacing (mm)	0.453 (0.366–0.475)	0.502 (0.372–0.530)	0.44
Cortical porosity (%)	3.4 (2.4–3.4)	3.0 (3.3–3.6)	0.86
Cortical thickness (mm)	0.894 (0.718–1.010)	0.894 (0.661–1.065)	0.83
Bone strength			
Bone stiffness (N/mm)	86,548 (59,060–117,353)	84,632 (71,231–94,599)	0.96
Estimated failure load (N)	4293 (3675–4814)	4370 (3000–6000)	0.92

Data are presented as median with 25–75 % IQR

Geometry, vBMD, and micro-architecture by HR-pQCT

Bone geometry, volumetric BMD, micro-architecture, and bone strength were obtained by the HR-pQCT, and data are shown in Table 2 for distal radius and in Table 3 for distal tibia. There were no differences between MF patients and healthy controls in any of the measured parameters in both radius and tibia. At the tibia, there was a trend towards an increase in trabeculae number (*P* = 0.09) and decrease in trabeculae thickness (*P* = 0.14) and trabeculae spacing (*P* = 0.10) in MF patients in comparison to controls (Table 3).

Similarly, total bone stiffness and estimated failure load were not significantly different between MF patients and the healthy controls in either of the skeletal sites examined (Tables 2, 3). It was not possible to obtain sufficient image quality in two patients at radial site and in one patient at tibial site due to motion artefact.

Biochemical analysis

Biochemical measurements amongst MF patients revealed decreased haemoglobin and elevated lactate dehydrogenase, whilst leucocytes and platelets were within normal range (Table 1).

Table 3 Bone geometry, volumetric bone mineral density, and micro-architecture by HR-pQCT; failure load by FEA in patients with myelofibrosis (MF) and healthy controls in distal tibia

	MF patients (<i>N</i> = 17)	Healthy controls (<i>N</i> = 17)	<i>P</i> value
Bone geometry			
Total area (mm ²)	832.8 (668.7–961.2)	842.7 (716.8–985.2)	0.69
Cortical area (mm ²)	116.5 (92.4–146.0)	118.1 (87.1–151.7)	0.90
Trabecular area (mm ²)	708.8 (568.5–804.4)	715.4 (619.7–838.4)	0.69
Volumetric bone mineral density			
Total density (mg/cm ³)	273.8 (236.8–301.9)	270.1 (218.5–305.1)	0.82
Cortical density (mg/cm ³)	821.0 (795.3–854.2)	789.3 (769.1–831.5)	0.21
Trabecular density (mg/cm ³)	175.1 (156.2–201.5)	170.7 (145.3–185.1)	0.82
Micro-architecture			
Trabecular (BV/TV (%))	0.146 (0.130–0.168)	0.142 (0.121–0.154)	0.81
Trabecular number (mm ⁻¹)	2.16 (1.86–2.34)	1.90 (1.75–2.06)	0.09
Trabecular thickness (mm)	0.069 (0.063–0.075)	0.075 (0.068–0.087)	0.14
Trabecular spacing (mm)	0.418 (0.362–0.458)	0.461 (0.398–0.495)	0.10
Cortical porosity (%)	0.069 (0.050–0.081)	0.085 (0.054–0.119)	0.21
Cortical thickness (mm)	1.115 (1.009–1.243)	1.142 (0.955–1.328)	0.76
Bone strength			
Bone stiffness (N/mm)	221,110 (177,429–251,559)	217,939 (149,006–254,118)	0.89
Estimated failure load (N)	11265 (8903–13,065)	10,995 (7773–12,689)	1.00

Data are presented as median with 25–75 % IQR

Thyroid-stimulating hormone, ionized calcium, phosphate, creatinine, and alkaline phosphatase were within reference values. PTH was marginally above reference values amongst 6 patients and the level of 25-OH-vitamin D was below reference values amongst 10 patients.

Levels of biochemical bone turnover markers are listed in Table 1. MF patients had significantly higher levels of P1NP compared to the reference level ($P < 0.001$), whilst the remaining markers bALP, OC, and CTX were within reference values.

Discussion

This study is the first assessing the bone structure with HR-pQCT in a small cohort of patients diagnosed with myelofibrosis by a direct comparison to a matched group of healthy volunteers. Our data revealed no differences between patients and healthy volunteers with regard to bone geometry, volumetric bone mineral density, and micro-structure measured at distal tibia and distal radius. Estimated bone stiffness and bone strength were similar between MF patients and healthy controls.

DXA BMD

Previous studies of BMD amongst patients with MF have been mainly small cross-sectional studies including MF patients compared with a matched group from the background population, with up to nine participants or case

reports [7, 8]. Diamond et al. [8] reported increased bone mass in a series of four MF patients compared to normative data from the Australian population. Mellibovsky et al. [7] showed in a study that hip BMD was above average in eight of nine patients with MF when compared with individuals matched for height and age. The same study also observed that MF patients in disease stages one or two had lower BMD than those with stage three. Secondary osteoporosis and osteolytic lesions are extremely rare findings in myelofibrosis, but have been described in case reports [10, 32, 33].

In our study the areal bone mineral density, estimated by DXA, as well as the volumetric bone mineral density assessed using HR-pQCT in patients with myelofibrosis were comparable with the background population, although osteosclerotic manifestations were observed in the bone marrow biopsies. Elevated BMD in the spine may reflect an artefact from degenerative conditions, as seen in the elderly populations [34]. Artefact may also partly explain the splenomegaly reported associated with elevated BMD at lumbar site [35, 36]. This was found in a patient with Gaucher's disease who also had coexistent low hip BMD, possibly reflecting the high glycolipid load or secondary calcification of the spleen [35]. Of note Gaucher's disease often results in bone manifestations where increased risk of osteoporosis and fractures is a common feature [37]. Splenomegaly is an almost universal finding in MF and therefore spine BMD may not be as accurate as hip BMD in our patient group. In situations with discrepancy between BMD measurements of spine and hip

in MF patients, an artefact from splenomegaly must be considered.

CMPNs and bone pathophysiology

Patients with systemic mastocytosis (SM), another rare chronic myeloproliferative neoplasm has decreased bone mass and increased risk of osteoporosis [38]. Conversely, osteosclerotic changes amongst patients with SM have also been described. In spite of this, these CMPN type patients were reported to have increased bone mass with increased trabecular volume, increased cortical thickness and narrowing of marrow spaces, and osteosclerosis and this was associated with a poorer disease outcome [38–40]. The pathogenesis of the affected bone metabolism in SM is unknown. The neoplastic mast cell has been suggested to be involved as well as inflammatory mediators such as IL-6 may contribute to the bone pathophysiology [41, 42]. The mast cell and its mediator histamine do not play an important role in the classical CMPNs. A common feature between SM and the classical CMPNs may however be the chronic inflammation of the bone marrow which again may affect bone metabolism. Elevation of IL-6 is also found amongst the classic CMPN patients [43, 44]. Possibly, chronic inflammation may cause a bone loss in CMPN leading to an increased risk of fractures but the loss may be masked by an elevation of BMD which is caused by osteosclerosis. Of note, glucocorticoids may be used as a symptom-directed therapy in SM, whereas treatment of the classical CMPN patients does not include corticosteroids or other agents known to affect bone metabolism [45].

Bone remodelling and bone turnover markers

Bone turnover in our study was assessed with biochemical markers, reflecting the global bone status, as recommended by International Osteoporosis Foundation and International Federation of Clinical Chemistry and Laboratory Medicine in clinical studies [46]. BTM in MF patients have been investigated in several studies [7, 8, 47, 48]. In the study by Mellibovsky et al. [7] osteocalcin was within normal range in the MF patients, consistent with our results. Diamond et al. [8] found elevated markers of bone formation; alkaline phosphatase and bone gla-protein as well as elevated markers of bone resorption; carboxy telepeptidase (ICTP); and deoxypyridinoline in their case report of 4 MF patients. PINP is a direct marker for collagen synthesis, and therefore used as a proxy marker of bone formation in clinical studies. However, this marker is likely to also reflect the neoplastic driven bone marrow fibrosis in MF. Patients with myelofibrosis have excess collagen type I and type III fibres in the bone marrow [2, 49] which may explain the significant elevation of PINP in our study. Studies have shown

that pro-collagen III peptide (P3NP)—a marker of collagen 3 syntheses—is elevated in CMPN patients compared with controls, and that the level is highest in MF patients. Also P3NP increases with the degree of reticulin fibrosis, and thus correlates with disease activity in patients with CMPN [47, 48]. Furthermore, a marker of collagen degradation, carboxy-terminal telopeptide of type I collagen (S-ICTP), is significantly higher in MF patients compared with controls [48].

Limitations

An overlap exists within the different CMPN entities in ICD-10 and diagnostic misclassification can occur [50]. In this study, all MF diagnoses were re-evaluated by review of both the medical files and the bone marrow biopsies. Since the bone marrow biopsies were not performed at the time of the scanning, some patients may have progressed in fibrosis grade or developed osteosclerosis since the last bone marrow biopsy. A possible explanation for the lack of differences may be that the peripheral scanning measurements are underestimating the actual new bone formation and the biomechanical properties in the central skeleton, including the location of haematopoietic tissue, even though it has been shown in healthy individuals that distal measurements by HR-pQCT of radius and tibia are associated with bone density, micro-structural, and mechanical properties of the central skeleton [51]. However, at the present time the high resolution of the HR-pQCT cannot be obtained when measuring central anatomical locations.

Previous, associations between fracture risk and estimated failure load have been demonstrated [52]. In the perspective of increased fracture risk found amongst other types of CMPN patients, none of the structural parameters in this study are indicating an increased risk of fractures in MF patients [11]. The reason for this potential discrepancy is unknown, but since MF is a rare disease with an incidence ranging from 0.1 to 1.0 per 100,000 person years and a median survival of 6.6 years [53–55], we may have had inadequate statistical power to detect differences between groups. Figure 1 shows a possible increased aBMD in the spine of MF patients relative to control. Given the modest number of patients included, it is possible that aBMD are higher in MF patients, and that this could have been demonstrated in a larger patient population. The distribution of the patients in DIPSS plus categories did not allow comparison of aBMD or other measures across categories.

Perspectives and conclusion

A collaborative study is to be considered in the future to achieve a larger patient cohort, but is restricted by the availability of HR-pQCT scans at present. In a cross-sectional

study design, the structural changes expected along with progression of the MF are not evaluated. Mellibovsky et al. [7] suggest that the coupling between progression of MF and BMD could constitute a non-invasive method for follow-up of MF. Ultimately, non-invasive bone assessment in addition with biochemical markers of fibrosis may prove to be a way of monitoring disease and treatment response amongst patients with MF in the future to minimize the number of invasive bone marrow biopsies.

In summary, data from HR-pQCT in a cohort of 18 patients with myelofibrosis regarding bone geometry, bone mineral density, and micro-architecture were comparable with the background population, despite manifestations of osteosclerosis observed in bone marrow biopsies of the patients. PINP were significant elevated and may be a marker of formation of fibrosis to include in further studies.

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Conflict of interest The authors report no conflict of interest.

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