

Letter to the Editor

Serum levels of bone cytokines are increased in indolent systemic mastocytosis associated with osteopenia or osteoporosis

To the Editor:

Mastocytosis is characterized by pathologic accumulation of mast cells (MCs) related to activating *KIT* mutations.¹ Patients with systemic mastocytosis (SM) usually show multifocal infiltrates of MCs in the bone marrow (BM). Apart from anaphylaxis, one of the most frequent and debilitating symptoms in SM is osteoporosis.²⁻⁸ Although it is likely that the MC infiltrates in BM either directly or indirectly affect skeletal structures, the pathogenesis of mastocytosis-associated osteoporosis remains poorly understood.

Within all categories of SM, more than half of the patients show skeletal involvement, including osteopenia, osteoporosis, osteolytic lesions, and osteosclerosis.²⁻⁸ MC mediators that have been discussed to affect bone remodeling include histamine, heparin, and IL-6. Current therapy of SM-related osteoporosis is largely experimental. Controlled clinical trials investigating the efficacy of novel targeted therapies such as antibodies against receptor activator of nuclear factor kappa-B ligand (RANKL), dickkopf-1 (Dkk-1), and sclerostin (SOST) are still missing.

In the past decade, our knowledge of bone biology increased tremendously.⁹ A series of new cellular and molecular players has been identified that coordinate the balance between bone-forming osteoblasts and bone-degrading osteoclasts in a highly complex manner. Among these processes, the signaling pathway involving RANKL, receptor activator of nuclear factor kappa-B (RANK), and osteoprotegerin (OPG) plays a major role. RANKL is produced by osteoblasts and binds to its receptor RANK expressed on osteoclasts. This process is inhibited by OPG, a naturally occurring RANKL antagonist also produced by osteoblasts. Interestingly, elevated serum levels of OPG were recently observed in mastocytosis.⁴ The Wnt/ β -catenin pathway is another crucial mechanism controlling the differentiation of osteoblasts. Dkk-1 and SOST modulate this pathway through binding to the Wnt receptor.

In the present study, we sought to investigate the pathophysiology of skeletal involvement in mastocytosis. In particular, we aimed to explore whether key cytokines of bone metabolism are altered in serum of patients with SM and whether MCs are able to produce these cytokines.

Bone mineral density (BMD) data of lumbar spine and femoral neck were collected in 56 patients with different categories of SM, classified according to established World Health Organization criteria (patient group 1; $n = 56$; see [Table E1](#) in this article's Online Repository at www.jacionline.org).¹ Absolute BMD values were converted into T scores, defining a T score below -2.5 SD as osteoporosis and below -1.0 SD and equal or above -2.5 SD as osteopenia.

Out of patient group 1, a representative number of patients with indolent SM (ISM) and osteopenia or osteoporosis of the lumbar spine was selected (patient group 2; $n = 21$; [Table E1](#)). In this subgroup as well as in 10 healthy controls, serum levels of bone cytokines, including IL-6, RANKL, OPG, Dkk-1, and SOST, as well as of tryptase, calcium (Ca), parathyroid hormone, and vitamin D were determined as detailed in this article's [Methods](#) section in the Online Repository at www.jacionline.org.

Furthermore, BM biopsies from patients with SM were stained with antibodies against bone cytokines by immunohistochemistry and immunofluorescence (see this article's [Methods](#) section in the Online Repository). In addition, bone markers were measured in cell culture supernatants of different cell lines.

In line with previous reports,²⁻⁸ patient group 1 representing 56 unselected adult patients with different categories of SM showed a clearly decreased BMD ([Fig 1, A and B](#), and [Table E1](#)). The decrease in lumbar spine ([Fig 1, A](#)) was more pronounced than that in femoral neck ([Fig 1, B](#)). Overall, 60.7% of the patients showed osteopenia and 10.7% osteoporosis ([Table E1](#)). In our patient population, there was no significant difference between male and female patients ([Fig E1, A and B](#)) and between older and younger patients ([Fig E1, C and D](#)). Most patients with ISM had osteopenia and osteoporosis, whereas patients with advanced SM categories often showed normal or increased BMD values ([Fig E1, E and F](#)).

To investigate the mechanism of skeletal involvement in mastocytosis, we determined several key markers of bone turnover in a largely homogeneous subgroup of 21 patients with ISM with osteopenia or osteoporosis (patient group 2; [Fig 1, C to H](#); [Table E1](#); see [Fig E2, A and B](#), and [Table E2](#) in this article's Online Repository at www.jacionline.org). As anticipated, patients with ISM showed significantly increased tryptase ([Fig 1, C](#)) and IL-6 ([Fig 1, D](#)) levels compared with healthy controls. Interestingly, serum levels of RANKL ([Fig 1, E](#)) and OPG ([Fig 1, F](#)) were significantly increased in patients with ISM than in controls, suggesting the involvement of the RANKL/RANK/OPG pathway in mastocytosis-related osteoporosis. Moreover, levels of SOST were significantly enhanced ([Fig 1, H](#)), indicating that the Wnt pathway may additionally be affected. We did not observe altered levels of Dkk-1 ([Fig 1, G](#)).

Levels of Ca, parathyroid hormone, and vitamin D were comparable between patients with ISM and controls ([Fig E2, C](#); see [Table E3](#) in this article's Online Repository at www.jacionline.org); however, most patients received Ca supplementation and vitamin D at the time of serum collection, in addition to antihistamines, which limits the interpretation of these results (see [Table E4](#) in this article's Online Repository at www.jacionline.org).

To define the cellular source of bone cytokines, serial sections of BM biopsies from patients with SM were stained with antibodies against tryptase, RANKL, and OPG ([Fig 1, I and J](#)). We observed clear colocalization of RANKL- and OPG-positive cells with tryptase-positive MCs, demonstrating that MCs in SM produce RANKL and OPG. To confirm the production of bone cytokines by MCs, supernatants of the human MC line HMC1 were investigated ([Fig 1, K](#)). The osteoblast-like cell lines SaOs and MG63 served as positive controls. In 2 HMC1 subclones (HMC1.1 and HMC1.2), we detected significant levels of RANKL, OPG, and SOST. Of note, production of all 3 cytokines was more pronounced in HMC1.2, which carries the mastocytosis-associated mutation *KIT*^{D816V}.

Taken together, our results show for the first time that RANKL, OPG, and SOST are significantly increased in ISM associated with osteopenia or osteoporosis and that MCs in SM represent a cellular source of RANKL and OPG. As illustrated in [Fig 2](#), bone cytokines produced by MCs may modulate the bone turnover in various ways.⁹ IL-6 and RANKL may induce activation and differentiation of osteoclasts. OPG may inhibit maturation of osteoclasts, which would

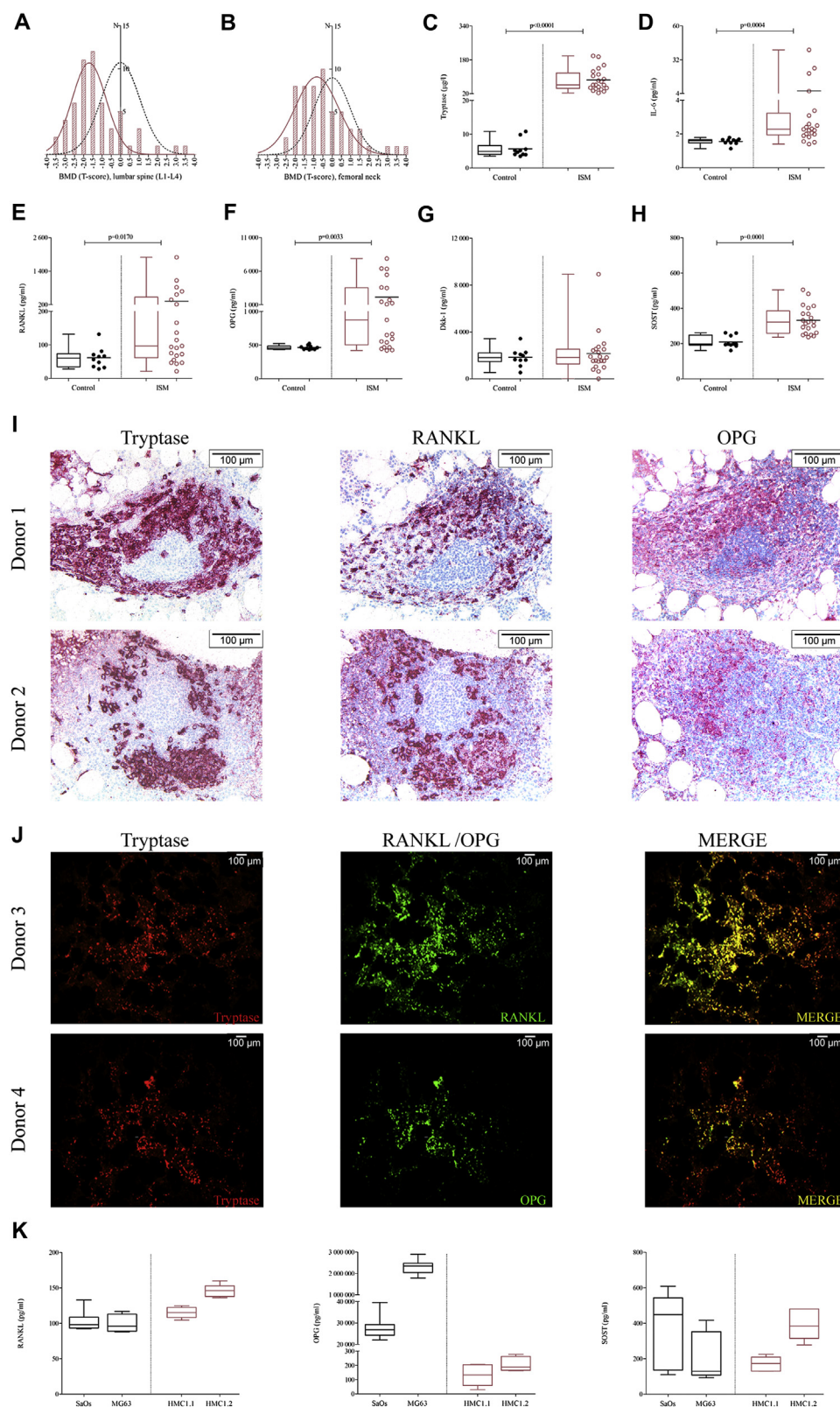


FIG 1. A-B, Overlay of the normal BMD distribution in controls (black dotted line) against the observed BMD in patients with SM (patient group 1; red line) at lumbar spine (Fig 1, A) and femoral neck (Fig 1, B). Bars show the number of patients per T score. C-H, Tryptase (Fig 1, C), IL-6 (Fig 1, D), RANKL (Fig 1, E), OPG (Fig 1, F), Dkk-1 (Fig 1, G), and SOST (Fig 1, H) serum levels in patients with ISM (patient group 2) and controls. Data are presented as box plots (minimum to maximum) with medians (left) and scatter dot plots with means (right). I-J, Expression of tryptase, RANKL, and OPG in the BM of patients with SM analyzed by immunohistochemistry (Fig 1, I) and immunofluorescence (Fig 1, J). K, RANKL, OPG, and SOST levels in supernatants of cultured MCs (HMC1.1 and HMC1.2) compared with osteoblast cell lines (SaOs, MG63).

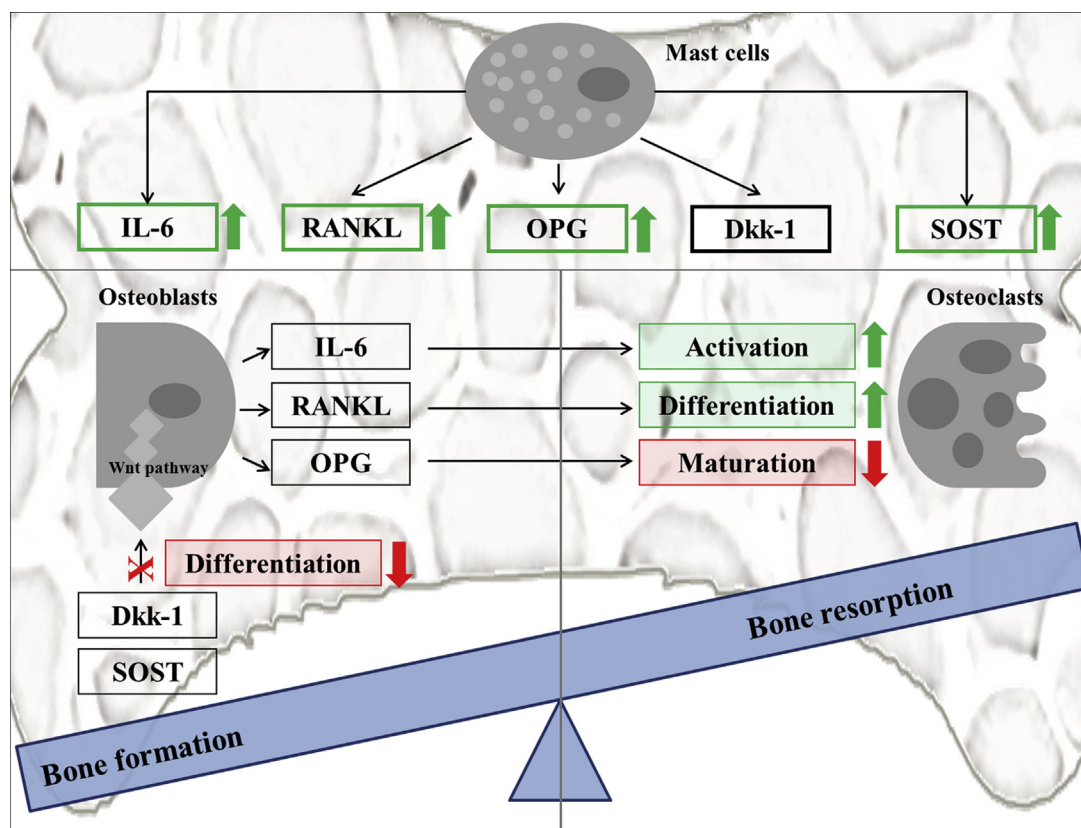


FIG 2. Mechanism of bone destruction in mastocytosis-associated osteoporosis. MC-derived bone cytokines, including IL-6, RANKL, OPG, Dkk-1, and SOST, could directly affect functions of osteoblasts and osteoclasts, shifting the complex balance of bone turnover toward increased bone resorption and decreased bone formation.

further promote bone resorption. However, SOST may block differentiation of osteoblasts via the Wnt pathway, which would decrease bone formation. Thus, the production of bone cytokines by MCs may shift the balance of bone turnover toward increased bone resorption as well as decreased bone formation. Our data provide a rationale for investigating RANKL, OPG, and SOST as potential diagnostic markers of SM-related osteoporosis. Moreover, our results suggest exploring these cytokines as novel therapeutic targets in mastocytosis with skeletal involvement.

We thank C. Berns (Department of Dermatology, University of Cologne) for excellent technical assistance.

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This work was supported by research grants from the German Research Council (DFG; CRC/SFB832, project A14), the German-Israeli Foundation for Scientific Research and Development (993/2008), and the Wilhelm Sander Foundation (1999.049.2) to K.H.

Disclosure of potential conflict of interest: K. Hartmann is a consultant in a Novartis clinical trial on mastocytosis; is (or recently was) a speaker for ALK-Abelló, Novartis, and Shire; and received research grants from Novartis. The rest of the authors declare that they have no relevant conflicts of interest.

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METHODS

BMD and classification of bone involvement

BMD measurement was performed by using dual-energy x-ray absorptiometry. Absolute BMD values were converted into *T* scores, defined as the number of SDs above or below the mean of the peak bone mass in subjects in a reference database matched by gender and ethnicity. A *T* score below -2.5 SD was defined as osteoporosis, below -1.0 SD and equal or above -2.5 SD as osteopenia, below 1.0 SD and equal or above -1.0 SD as normal, and above 1.0 SD as osteosclerosis.

Serum measurements in patients with ISM

The following measurements were performed with patient's serum collected during the last 10 years at the Department of Dermatology, University of Cologne, Cologne, Germany, and stored at -80°C . Tryptase was determined by using fluoroimmunoassay (ImmunoCAP Tryptase; Thermo Fisher Scientific, Uppsala, Sweden), and levels of bone metabolism markers were measured by using ELISA (IL-6, eBioscience, Frankfurt, Germany; RANKL, Usn Life Science, Inc, Wuhan, China; OPG, Usn Life Science, Inc; Dkk-1, R&D Systems, Wiesbaden, Germany; SOST, Cusabio Biotech Co, Wuhan, China), according to the manufacturer's guidelines.

Calcium levels were determined by using Calcium-Test Gen.2 (Roche Diagnostics, Mannheim, Germany). Calcium ions react with 5-nitro-5'-methyl-BAPTA under alkaline conditions to form a complex. This complex reacts in the second step with EDTA. The change in absorbance is directly proportional to the calcium concentration and is measured photometrically at a Roche Modular P800-Modul (Roche Diagnostics).

Parathyroid hormone (PTH) levels were measured by using Elecsys-PTH assay (Roche Diagnostics). PTH, a biotinylated monoclonal PTH-specific antibody, and monoclonal PTH-specific antibody labeled with a ruthenium complex form a sandwich complex. After the addition of streptavidin-coated microparticles, the complex binds to the solid phase via interaction of biotin and streptavidin. The reaction mixture is transferred to the measuring cell, where the microparticles are magnetically captured onto the surface of the electrode. Application of a voltage to the electrode then induces chemiluminescent emission, which is measured by using a photomultiplier (Roche Modular E170-Modul, Roche Diagnostics).

25-Hydroxyvitamin D (vitamin D total) levels were measured by using Elecsys-Vitamin D Total assay (Roche Diagnostics). By incubating the sample with pretreatment reagents, bound vitamin D is released from the vitamin D-binding protein. Thereafter, the vitamin D-binding protein is labeled with ruthenium and a complex between vitamin D and the ruthenylated vitamin D-binding protein is formed. After the addition of streptavidin-coated microparticles and vitamin D labeled with biotin, unbound ruthenium-labeled vitamin D-binding proteins are marked. A complex consisting of the ruthenylated vitamin D-binding protein and the biotinylated vitamin D is formed and binds to the solid phase via interaction of biotin and streptavidin. The reaction mixture is transferred to the measuring cell, where the microparticles are magnetically captured onto the surface of the electrode. Application of a voltage to the electrode then induces chemiluminescent emission, which is measured by using a photomultiplier (Roche Modular E170-Modul, Roche Diagnostics).

Immunohistochemistry and immunofluorescence

For analysis of expression of RANKL and OPG protein in MC infiltrates of BM, serial sections of paraffin-embedded BM biopsies from patients with SM were stained with specific antibodies against tryptase (Dako, Carpinteria, Calif), RANKL (Abcam, Cambridge, United Kingdom), and OPG (antibodies-online GmbH, Aachen, Germany).

Sections of paraffin-embedded BM biopsies were also analyzed by double immunofluorescence staining using the Vectastain ABC-kit (Vector Laboratories, Inc, Burlingame, Calif). Sections were pretreated for antigen retrieval and permeabilization (Vector Laboratories, Inc) before incubation with rabbit anti-RANKL (Abcam) or anti-OPG antibodies (antibodies-online GmbH) overnight at 4°C . Fluorescein isothiocyanate-coupled goat antirabbit antibodies were added as secondary antibodies, and rabbit IgG antibody served as isotype control. Mouse antitryptase antibody (Abcam), horse antimouse biotinylated antibody, and avidin-linked Texas red dye were applied subsequently. Mouse IgG₁ (R&D Systems GmbH) was used for isotype controls. Analysis was carried out with Zeiss Axiocam and documented with Axiovision software (Carl Zeiss, Microimaging GmbH, Göttingen, Germany).

Cell lines and measurement of bone cytokines in cell culture supernatants

For *in vitro* experiments, the human osteoblast-like cell lines SaOs (established by J. Fogh, 1973) and MG63 (established by A. Billiau, 1977) were maintained in Dulbecco's modified Eagle medium ($1\times$; Gibco, Invitrogen, Karlsruhe, Germany) containing 10% FBS (Biochrom AG, Berlin, Germany), 2 mM L-glutamine (Biochrom AG), 100 U/mL penicillin (Biochrom AG), and 100 $\mu\text{g/mL}$ streptomycin (Biochrom AG). The 2 subclones of the human mast cell line HMC1 (HMC1.1 and HMC1.2) were kindly provided by Dr Joseph H. Butterfield (Mayo Clinic, Rochester, Minn) and cultured in Isocove's modified Dulbecco's medium ($1\times$; Gibco, Invitrogen) containing 10% FBS (Biochrom AG), 2 mM L-glutamine (Biochrom AG), 50 μM 2-mercaptoethanol (Gibco, Invitrogen), 100 U/mL penicillin (Biochrom AG), and 100 $\mu\text{g/mL}$ streptomycin (Biochrom AG). The subclone HMC1.1 carries 1 *KIT* mutation (V560G), whereas the subclone HMC1.2 carries 2 *KIT* mutations (V560G and D816V). All cell cultures were maintained at 37°C in 5% CO_2 in a humidified atmosphere.

For measurement of bone cytokines in the cell lines SaOs, MG63, HMC1.1, and HMC1.2, 1×10^7 cells were incubated in 10 mL of the appropriate medium for 72 hours. Thereafter, supernatants were collected and frozen at -20°C for ELISA measurements (RANKL, Usn Life Science, Inc; OPG, Usn Life Science Inc; SOST, Bio-medical Assay, Beijing, China), according to the manufacturer's guidelines. In 5 separate experiments, each sample was measured twice.

Statistical analysis

Statistical analysis was performed with GraphPad Prism (Version 5.01) software by using the nonparametric Mann-Whitney *U* test. A *P* value of less than .05 was considered statistically significant.

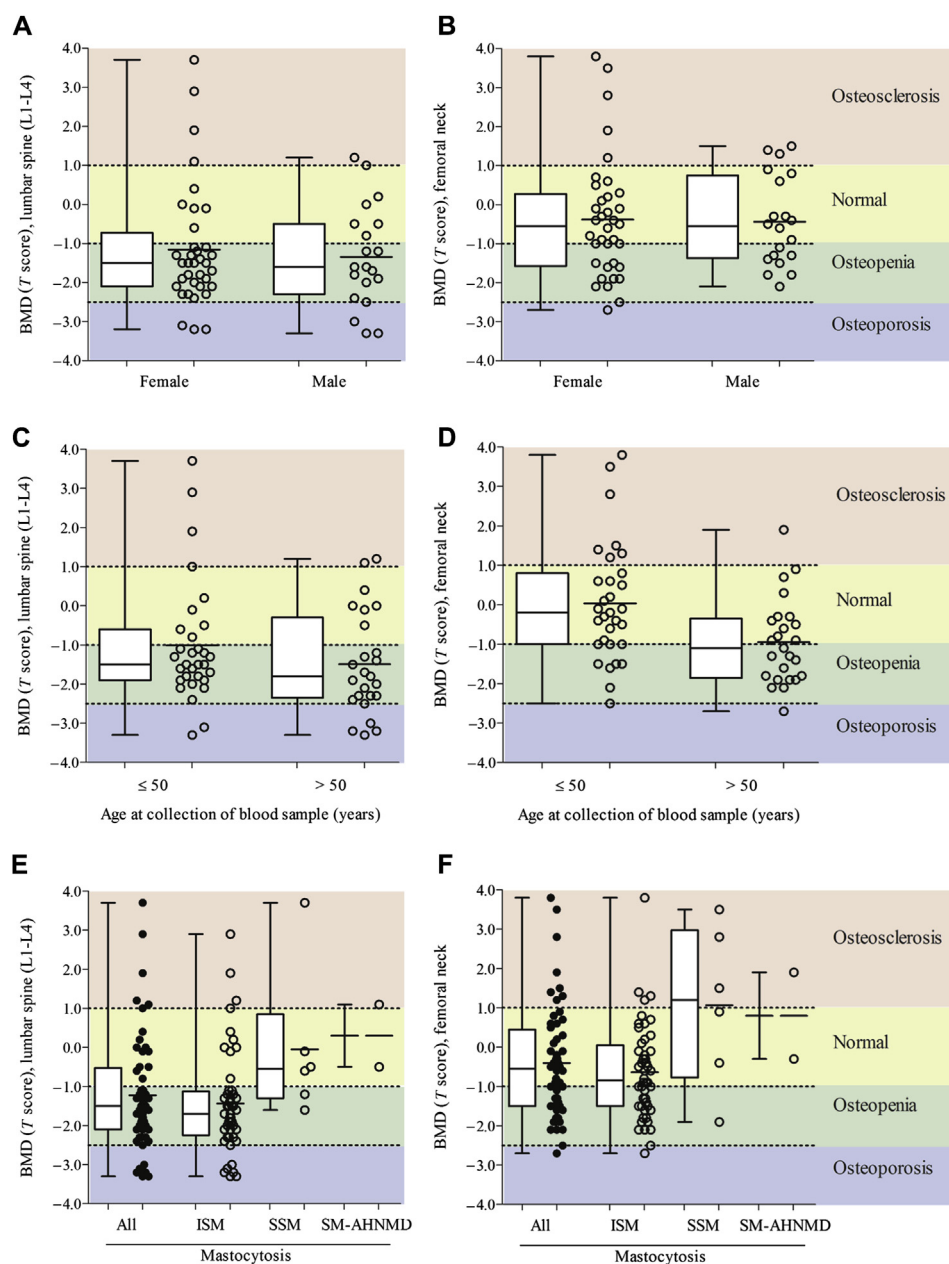


FIG E1. A-B, BMD of lumbar spine (Fig E1, A) and femoral neck (Fig E1, B) are comparable in female ($n = 36$) and male ($n = 20$) patients with SM (patient group 1; $n = 56$). C-D, BMD of lumbar spine (Fig E1, C) and femoral neck (Fig E1, D) are comparable in patients with SM younger than 50 years ($n = 31$) and older than 50 years ($n = 25$). E-F, BMD of lumbar spine (Fig E1, E) and femoral neck (Fig E1, F) in different categories of SM. Data are presented as box plots (minimum to maximum) with medians (left) and scatter dot plots with means (right). Colors illustrate scoring for bone disease (blue, osteoporosis; green, osteopenia; yellow, normal; beige, osteosclerosis). AHNMD, Associated hematologic non-MC lineage disease; SSM, smoldering SM.

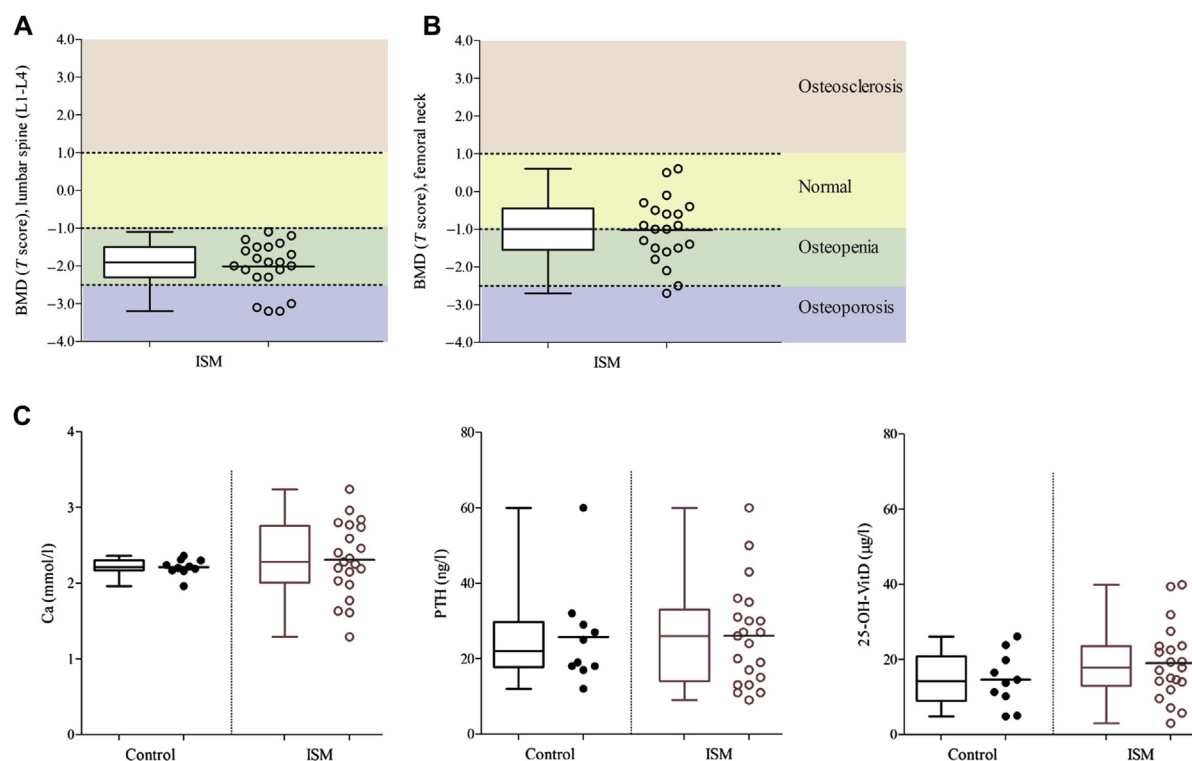


FIG E2. A-B, BMD of lumbar spine (Fig E2, A) and femoral neck (Fig E2, B) in patient group 2 (ISM subgroup; $n = 21$). Data are presented as box plots (minimum to maximum) with medians (*left*) and scatter dot plots with means (*right*). Colors illustrate scoring for bone disease (*blue*, osteoporosis; *green*, osteopenia; *yellow*, normal; *beige*, osteosclerosis). C, Calcium, PTH, and vitamin D in patient group 2 (ISM subgroup; $n = 21$) and healthy controls (control; $n = 10$). Data are presented as box plots (minimum to maximum) with medians (*left*) and scatter dot plots with means (*right*).

TABLE E1. Clinical characteristics of patients with mastocytosis and control subjects participating in the study

Parameters	Controls	Patients	
	All	Group 1, all	Group 2, ISM subgroup for serum marker measurements
All	10 (100)	56 (100)	21 (37.5)
Gender			
Male	3 (30.0)	20 (35.7)	5 (23.8)
Female	7 (70.0)	36 (64.3)	16 (76.2)
Age at collection of blood sample (y)			
Mean $\pm$ SD	35.5 $\pm$ 10.2	51.2 $\pm$ 12.6	50.2 $\pm$ 11.1
Range	25-55	22-80	32-72
Disease duration (y) (from diagnosis to blood collection)			
Mean $\pm$ SD	NA	8.7 $\pm$ 7.9	11.2 $\pm$ 9.1
Range	NA	0-32	0-32
Tryptase (μ g/L)			
Mean $\pm$ SD	5.7 $\pm$ 2.5	105.4 $\pm$ 102.5	84.8 $\pm$ 52.2
Range	3.5-10.8	9.6-501.0	23.5-199.0
Category of mastocytosis			
ISM	NA	48 (85.7)	21 (100)
SSM	NA	6 (10.7)	0 (0.0)
SM-AHNMD	NA	2 (3.6)	0 (0.0)
BMD			
Lumbar spine (L1-L4), <i>T</i> score			
Mean $\pm$ SD	NA	-1.2 $\pm$ 1.5	-2.0 $\pm$ 0.6
Range	NA	-3.3 to 3.7	-3.2 to -1.1
Lumbar spine (L1-L4), <i>Z</i> score			
Mean $\pm$ SD	NA	-0.9 $\pm$ 1.4	-1.7 $\pm$ 0.6
Range	NA	-3.0 to 3.3	-2.8 to -0.8
Femoral neck, <i>T</i> score			
Mean $\pm$ SD	NA	-0.4 $\pm$ 1.4	-1.0 $\pm$ 0.9
Range	NA	-2.7 to 3.8	-2.7 to 0.6
Femoral neck, <i>Z</i> score			
Mean $\pm$ SD	NA	0.0 $\pm$ 1.3	-0.6 $\pm$ 0.8
Range	NA	-2.0 to 3.5	-2.0 to 1.1
Category of bone disease			
Osteosclerosis (lumbar spine [L1-L4], <i>T</i> score: 4.0-1.0)	NA	6 (10.7)	0 (0.0)
Normal (lumbar spine [L1-L4], <i>T</i> score: 1.0 to -1.0)	NA	10 (17.9)	0 (0.0)
Osteopenia (lumbar spine [L1-L4], <i>T</i> score: -1.0 to -2.5)	NA	34 (60.7)	17 (81.0)
Osteoporosis (lumbar spine [L1-L4], <i>T</i> score: -2.5 to -4.0)	NA	6 (10.7)	4 (19.0)

AHNMD, Associated hematologic non-MC lineage disease; NA, not applicable; SSM, smoldering SM.

Values are n (%) unless indicated otherwise.

TABLE E2. BMD and bone cytokines in patient group 2 (ISM subgroup; n = 21)

Patient	BMD (T score)		Tryptase and bone cytokines					
	Lumbar spine (L1-L4)	Femoral neck	Tryptase (μg/L)	IL-6 (pg/mL)	RANKL (pg/mL)	OPG (pg/mL)	Dkk-1 (pg/mL)	SOST (pg/mL)
1	−1.8	−1.0	61.40	2.40	114.34	540.74	2721.60	367.50
2	−2.1	−1.5	40.00	2.22	67.95	419.26	1809.60	345.44
3	−1.9	−1.4	23.50	3.09	72.26	466.33	8929.60	419.11
4	−1.2	0.6	89.00	2.59	96.83	594.39	1489.60	481.60
5	−1.3	−1.5	48.40	2.28	562.96	1711.22	2993.60	368.90
6	−2.3	−1.6	34.00	2.50	75.14	6343.92	2513.60	401.48
7	−1.5	−0.4	155.00	2.22	136.41	424.63	2241.60	504.64
8	−1.7	−0.6	77.40	2.00	675.51	5462.54	4113.60	413.32
9	−2.1	0.5	104.00	1.98	214.57	663.41	625.60	260.95
10	−1.1	−0.1	81.80	1.90	166.17	1446.73	2417.60	321.44
11	−3.0	−1.8	195.00	20.63	376.88	3579.74	1.60	331.33
12	−1.9	−1.0	42.80	1.82	91.03	1080.43	1889.60	286.53
13	−1.4	−0.6	111.00	2.31	43.70	875.19	2529.60	301.00
14	−1.5	−2.5	61.80	25.03	1896.70	6390.57	977.60	323.47
15	−3.2	−2.7	47.00	3.38	46.54	1240.21	1537.60	256.05
16	−2.0	−1.3	125.00	40.19	823.48	3491.41	1553.60	313.02
17	−3.2	−0.9	199.00	1.56	93.93	555.17	1521.60	248.72
18	−2.3	−0.9	30.70	1.51	46.54	649.70	1889.60	248.40
19	−1.6	−0.5	51.90	5.95	1067.88	7883.08	1025.60	242.21
20	−3.1	−2.1	61.30	1.40	55.08	465.67	785.60	292.75
21	−2.0	−0.3	140.00	2.05	21.13	444.09	1521.60	236.46
All								
Mean ± SD	−2.0 ± 0.6	−1.0 ± 0.9	84.8 ± 52.2	6.1 ± 10.0	321.2 ± 464.9	2129.9 ± 2388.5	2147.1 ± 1800.8	331.6 ± 77.8
Range	−3.2 to −1.1	−2.7 to 0.6	23.5-199.0	1.4-40.2	21.1-1896.7	419.3-7883.1	1.6-8929.6	236.5-504.6

TABLE E3. BMD, calcium, and vitamin D in patient group 2 (ISM subgroup; n = 21)

Patient	BMD (T score)		Tryptase and bone modulators						
	Lumbar spine (L1-L4)	Femoral neck	Tryptase (μg/L)	Calcium (mmol/L)*	Calcium level	PTH (ng/L)†	PTH level	25-OH-Vit D (μg/L)‡	25-OH-Vit D level
1	−1.8	−1.0	61.40	1.29	Decreased	9.00	Decreased	17.10	Decreased
2	−2.1	−1.5	40.00	2.77	Increased	24.00	Normal	19.40	Decreased
3	−1.9	−1.4	23.50	1.63	Decreased	35.00	Normal	11.90	Decreased
4	−1.2	0.6	89.00	1.61	Decreased	31.00	Normal	3.00	Decreased
5	−1.3	−1.5	48.40	2.46	Normal	19.00	Normal	31.90	Normal
6	−2.3	−1.6	34.00	2.15	Decreased	17.00	Normal	17.80	Decreased
7	−1.5	−0.4	155.00	2.25	Normal	26.00	Normal	9.60	Decreased
8	−1.7	−0.6	77.40	2.03	Decreased	27.00	Normal	5.70	Decreased
9	−2.1	0.5	104.00	3.24	Increased	43.00	Normal	27.50	Decreased
10	−1.1	−0.1	81.80	2.59	Normal	15.00	Normal	39.90	Normal
11	−3.0	−1.8	195.00	2.80	Increased	50.00	Normal	21.90	Decreased
12	−1.9	−1.0	42.80	2.84	Increased	11.00	Decreased	39.40	Normal
13	−1.4	−0.6	111.00	2.20	Normal	30.00	Normal	23.60	Decreased
14	−1.5	−2.5	61.80	1.77	Decreased	36.00	Normal	7.10	Decreased
15	−3.2	−2.7	47.00	2.40	Normal	27.00	Normal	14.20	Decreased
16	−2.0	−1.3	125.00	2.28	Normal	30.00	Normal	14.00	Decreased
17	−3.2	−0.9	199.00	2.33	Normal	20.00	Normal	23.50	Decreased
18	−2.3	−0.9	30.70	2.19	Decreased	11.00	Decreased	19.30	Decreased
19	−1.6	−0.5	51.90	1.98	Decreased	13.00	Normal	15.00	Decreased
20	−3.1	−2.1	61.30	2.96	Increased	60.00	Normal	14.60	Decreased
21	−2.0	−0.3	140.00	2.74	Increased	13.00	Normal	22.50	Decreased
All, n (%)									
Mean ± SD	−2.0 ± 0.6	−1.0 ± 0.9	84.8 ± 52.2	2.3 ± 0.5	NA	26.0 ± 13.5	NA	19.0 ± 9.9	NA
Range	−3.2 to −1.1	−2.7 to 0.6	23.5-199.0	1.3-3.2	NA	9.0-60.0	NA	3.0-39.9	NA
Decreased	21 (100%)	11 (52.4%)	0 (0.0%)	NA	8 (38.1%)	NA	3 (14.3%)	NA	18 (85.7%)
Normal	0 (0.0%)	10 (47.6%)	0 (0.0%)	NA	7 (33.3%)	NA	18 (85.7%)	NA	3 (14.3%)
Increased	0 (0.0%)	0 (0.0%)	21 (100%)	NA	6 (28.6%)	NA	0 (0.0%)	NA	0 (0.0%)

25-OH-Vit D, 25-Hydroxyvitamin D; NA, not applicable.

*Normal range of calcium is 2.20 to 2.65 mmol/L.

†Normal range of PTH is 12.00 to 72.00 ng/L.

‡Normal range of 25-OH-Vit D is 30.00 to 70.00 μg/L.

TABLE E4. Antimediator therapy and osteopenia/osteoporosis therapy in patient group 2 (ISM subgroup; n = 21)

Patient	Antimediator therapy			Osteopenia/osteoporosis therapy	
	H ₁ antihistamines	H ₂ antihistamines	Disodium cromolyn	Calcium/vitamin D	Bisphosphonates
1	X	—	—	—	—
2	X	—	—	—	—
3	X	—	—	X	—
4	X	—	—	—	—
5	X	—	—	X	—
6	—	—	—	X	—
7	X	X	X	X	—
8	X	—	—	—	—
9	X	—	—	X	—
10	X	—	—	X	—
11	X	—	—	X	—
12	—	—	—	X	—
13	X	—	—	—	—
14	X	X	X	X	X
15	—	—	—	X	—
16	X	—	—	—	—
17	X	—	—	—	—
18	X	—	—	X	X
19	—	—	—	—	—
20	X	X	—	X	—
21	—	—	—	—	—
All, n (%)	16 (76.2)	3 (14.3)	2 (9.5)	12 (57.1)	2 (9.5)
Intake duration (mo) (until blood collection)					
Mean ± SD	65.2 ± 44.2	88.0 ± 64.2	106.0 ± 11.3	72.5 ± 42.8	69.5 ± 30.4
Range	4-146	19-146	98-114	12-131	48-91

X, Therapy received.