Original Article

Bone and Cytokine Markers Associated With Bone Disease in Systemic Mastocytosis

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What is already known about this topic? Mast cell mediators (eg, RANKL and IL-6) have been associated with bone loss in systemic mastocytosis, whereas evidence on the role of mediators other than tryptase in diffuse bone sclerosis is lacking.

What does this article add to our knowledge? Systemic mastocytosis with bone mass loss is associated with a proinflammatory cytokine profile, whereas diffuse bone sclerosis shows increased levels of biomarkers related to bone remodeling, in association with an immunosuppressive cytokine secretion profile.

How does this study impact current management guidelines? Routine measurement of bone remodeling markers should be recommended in all patients with systemic mastocytosis.

BACKGROUND: Mastocytosis encompasses a heterogeneous group of diseases characterized by tissue accumulation of clonal mast cells, which frequently includes bone involvement. Several cytokines have been shown to play a role in the pathogenesis of bone mass loss in systemic mastocytosis (SM), but their role in SM-associated osteosclerosis remains unknown.

OBJECTIVE: To investigate the potential association between cytokine and bone remodeling markers with bone disease in SM, aiming at identifying biomarker profiles associated with bone loss and/or osteosclerosis.

METHODS: A total of 120 adult patients with SM, divided into 3 age and sex-matched groups according to their bone status were studied: (1) healthy bone (n = 46), (2) significant bone loss (n = 47), and (3) diffuse bone sclerosis (n = 27). Plasma levels of cytokines and serum baseline tryptase and bone turnover marker levels were measured at diagnosis.

RESULTS: Bone loss was associated with significantly higher levels of serum baseline tryptase ($P = .01$), IFN-γ ($P = .05$), IL-1β ($P = .05$), and IL-6 ($P = .05$) versus those found in patients with healthy bone. In contrast, patients with diffuse bone sclerosis showed significantly higher levels of serum baseline tryptase ($P < .001$), C-terminal telopeptide ($P < .001$), amino-terminal propeptide of type I procollagen ($P < .001$), osteocalcin ($P < .001$), bone alkaline phosphatase ($P < .001$), osteopontin

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Abbreviations used
ASM- aggressive SM
BM- bone marrow
BMD- bone mineral density
BMM- bone marrow mastocytosis
BSAP- bone-specific alkaline phosphatase
C- cluster
CTX- C-terminal telopeptide of type I collagen
ISM- indolent SM
MC- mast cell
OPG- osteoprotegerin
OPN- osteopontin
OSM- oncostatin M
PINP- amino-terminal propeptide of type I procollagen
PTH- parathormone
RANKL- receptor activator of nuclear factor κ-B ligand
SM- systemic mastocytosis

($P < .01$), and the C-C Motif Chemokine Ligand 5/RANTES chemokine ($P = .01$), together with lower IFN-γ ($P = .03$) and RANK-ligand ($P = .04$) plasma levels versus healthy bone cases.

CONCLUSIONS: SM with bone mass loss is associated with a proinflammatory cytokine profile in plasma, whereas diffuse bone sclerosis shows increased serum/plasma levels of biomarkers related to bone formation and turnover, in association with an immunosuppressive cytokine secretion profile. **(J Allergy Clin Immunol Pract 2023;11:1536-47)**

Key words: *Systemic mastocytosis; Cytokines; Biomarkers; Bone metabolism; Osteoporosis; Osteosclerosis; Bone disease*

INTRODUCTION

Systemic mastocytosis (SM) includes a heterogeneous group of rare diseases, characterized in common by the accumulation of clonal mast cells (MCs) in tissues, such as the skin, the gastrointestinal tract, and the bone marrow (BM).[1] In a significant fraction of patients with SM, bone is also affected,[2,3] with an overall prevalence of osteoporosis ranging between 10% and 38%, among adult SM patients.[4,5] However, differences in the prevalence and subtypes of bone disease are observed among the distinct diagnostic categories of SM. Patients with aggressive SM (ASM) display a higher risk of more severe bone involvement, typically with osteolytic lesions,[2] whereas patients who have BM mastocytosis (BMM) associated with anaphylaxis in the absence of skin lesions tend to display a higher prevalence of osteoporosis.[4,6] In turn, diffuse bone sclerosis is typically related to ASM cases, among whom it affects up to one-third of the patients (Alvarez-Twose et al, unpublished observations, 2023) versus only 3% to 10% of other patients with SM, including 6% of adults with indolent SM (ISM),[3] the underlying pathogenic mechanisms remaining to be elucidated.[4,5,7,8] In contrast to osteolysis, which may be secondary to local MC infiltration, bone density loss and diffuse bone sclerosis might be associated with an increased release of different patterns of MC mediators by *KIT*-mutated MCs, which inhibit the formation of the (bone) extracellular matrix and/or decrease bone resorption, respectively.[7]

In line with all the above, previous studies have identified several different MC mediators to play a key role in bone metabolism. Thus, histamine,[9,10] tryptase,[11-14] prostaglandins, and multiple cytokines[15,16] are currently known to promote osteoclastogenesis and to play an important role in bone resorption in different disease conditions. In mastocytosis, high levels of both receptor activator of nuclear factor κ-B ligand (RANKL) and interleukin (IL-) 6 have been associated with bone loss.[17] In turn, the presence of osteosclerosis has been associated with increased serum baseline tryptase (sBT) levels in SM,[8,18,19] whereas evidence about the role of other MC mediators in the pathogenesis of osteosclerosis is lacking.

Here, we investigated the potential association between bone remodeling markers and cytokines and bone disease caused by SM, through the identification of unique biomarker profiles of bone loss and osteosclerosis in the plasma/serum of adult patients with SM that might contribute to an earlier diagnosis and a better understanding of the pathogenesis of bone disease in SM.

METHODS

Study design

We performed a cross-sectional study based on 120 adult patients diagnosed with SM and followed at the Spanish Reference Center for Mastocytosis. Before entering the study, each patient provided his or her written informed consent to participate, after the study had been approved by the Ethics Committee of the Hospital Center of Toledo (Spain), according to the Declaration of Helsinki.

Patients' demographic, clinical, and laboratory data were retrospectively collected from their medical records. Diagnosis and classification of SM was retrospectively redefined on the basis of BM morphological,[20] histopathological, immunohistochemical,[21] immunophenotypic,[21] and molecular[22] criteria as defined by both the World Health Organization 2022[23,24] and the International Consensus Classifications.[25,26] A detailed description of diagnostic procedures is provided in the Methods section of this article's Online Repository at www.jaci-inpractice.org. For the purpose of this study, patients with SM were divided into 3 groups according to their bone disease status: (1) absence of bone disease, (2) significant bone density loss (T-score < 2.2), and (3) diffuse bone sclerosis (the specific criteria used are described below).

Patient and sample selection criteria

Patients ($n = 120$) were randomly selected (retrospectively) among those who had consulted at the Spanish Reference Center for Mastocytosis for up to 40 patients per each of the 3 bone disease patient groups. Inclusion criteria were (1) age above 18 years, (2) diagnosis of SM, (2) full clinical and laboratory evaluation performed at the Spanish Reference Center for Mastocytosis; and (4) plasma samples collected and frozen at −80ºC, before any cytoreductive medication. Patients who had been diagnosed with well-differentiated subvariants of SM[27] were excluded from the study. To avoid age differences among the groups, patients with severe osteopenia (T-score ≤ -2), either at the lumbar spine or at the femoral neck, were specifically selected for the bone density loss group.

Diagnosis of bone disease

All patients were submitted to bone mineral density (BMD) assessment through dual-energy X-ray absorptiometry. Those with

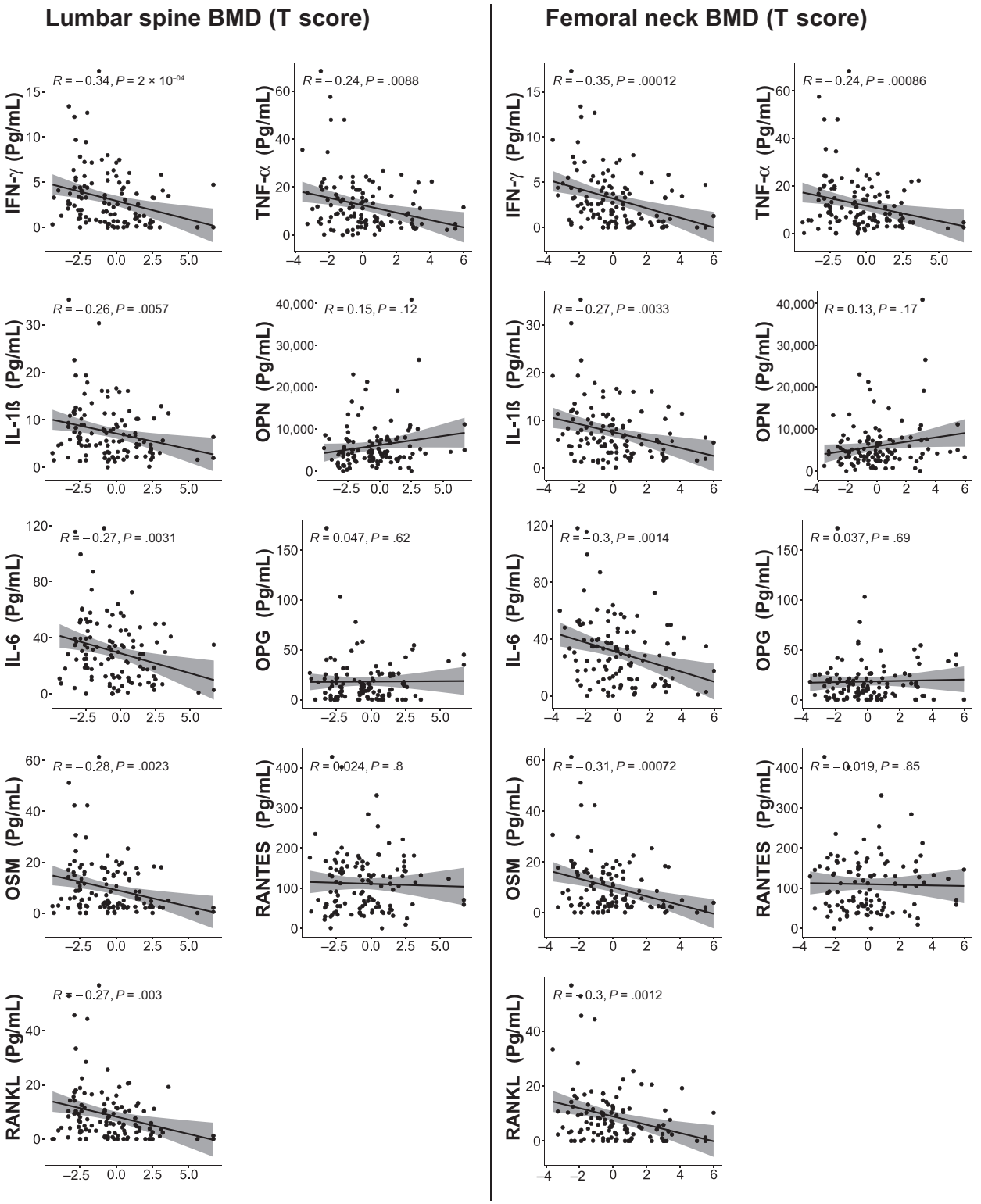

FIGURE 1. Correlation between serum/plasma biomarkers and both the lumbar spine (**A**) and the femoral neck (**B**) BMD T-scores, in SM. *OP*, Osteoporosis/severe osteopenia; *OS*, diffuse bone sclerosis; *Vit*, vitamin.

lumbar spine or femoral neck BMD T-score of −2 or less were classified as having significant bone loss, whereas those showing a BMD T-score above −1 were considered to have normal bone density. Diffuse bone sclerosis was defined as a significant increase in bone density observed in X-ray or computed tomography imaging.

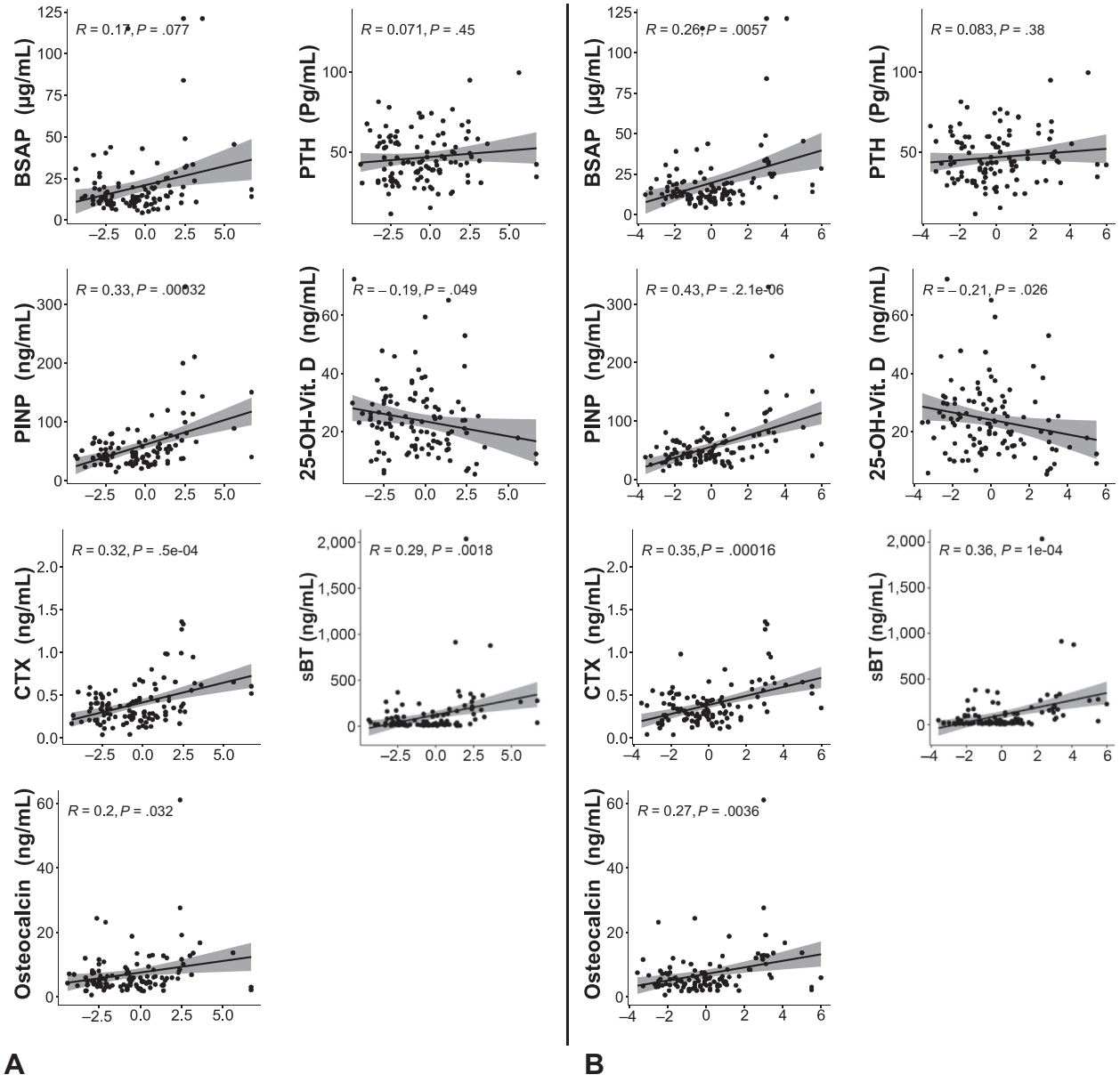

FIGURE 1. (continued)

Quantification of soluble bone markers and cytokine in plasma/serum markers

Plasma levels of IFN-γ, IL-1β, IL-6, oncostatin M (OSM), osteoprotegerin (OPG), RANKL, and tumor necrosis factor (TNF-) α were measured using a customized ProcartaPlex multiplex kit (eBioscience, Vienna, Austria). In turn, osteopontin (OPN) and C-C Motif Chemokine Ligand 5/RANTES were measured using the ProcartaPlex uniplex kits (eBioscience), assessed in a Bio-Plex MAGPIX Multiplex Reader (Bio-Rad, Hercules, Calif), strictly following the instructions of the manufacturer.

Data on other bone-related markers that had been previously measured in the same samples were collected from the patients' medical records. These included (1) amino-terminal propeptide of type I procollagen (PINP), parathormone (PTH), and C-terminal telopeptide of type I collagen (CTX), which were assessed by electrochemiluminescence (Cobas 8000 e602, Roche, Basel, Switzerland); (2) bone-specific alkaline phosphatase (BSAP) and vitamin D measured by chemiluminescence (Liaison XL, DiaSorin, Saluggia, Italy); and (3) osteocalcin, as determined by the Immulite 2000 immunoassay (Siemens Healthcare GmbH, Erlangen, Germany). Data on sBt (ImmunoCAP, Thermo Fisher Scientific, Uppsala, Sweden) were also collected from the patients' clinical records.

Statistical methods

For all continuous variables, median and interquartile range values were calculated, whereas for categorical variables frequencies were determined. The Mann-Whitney *U* or Kruskal-Wallis test and the χ^2 or Fisher's exact test were used to assess the statistical significance of differences observed between 2 or more than 2 groups,

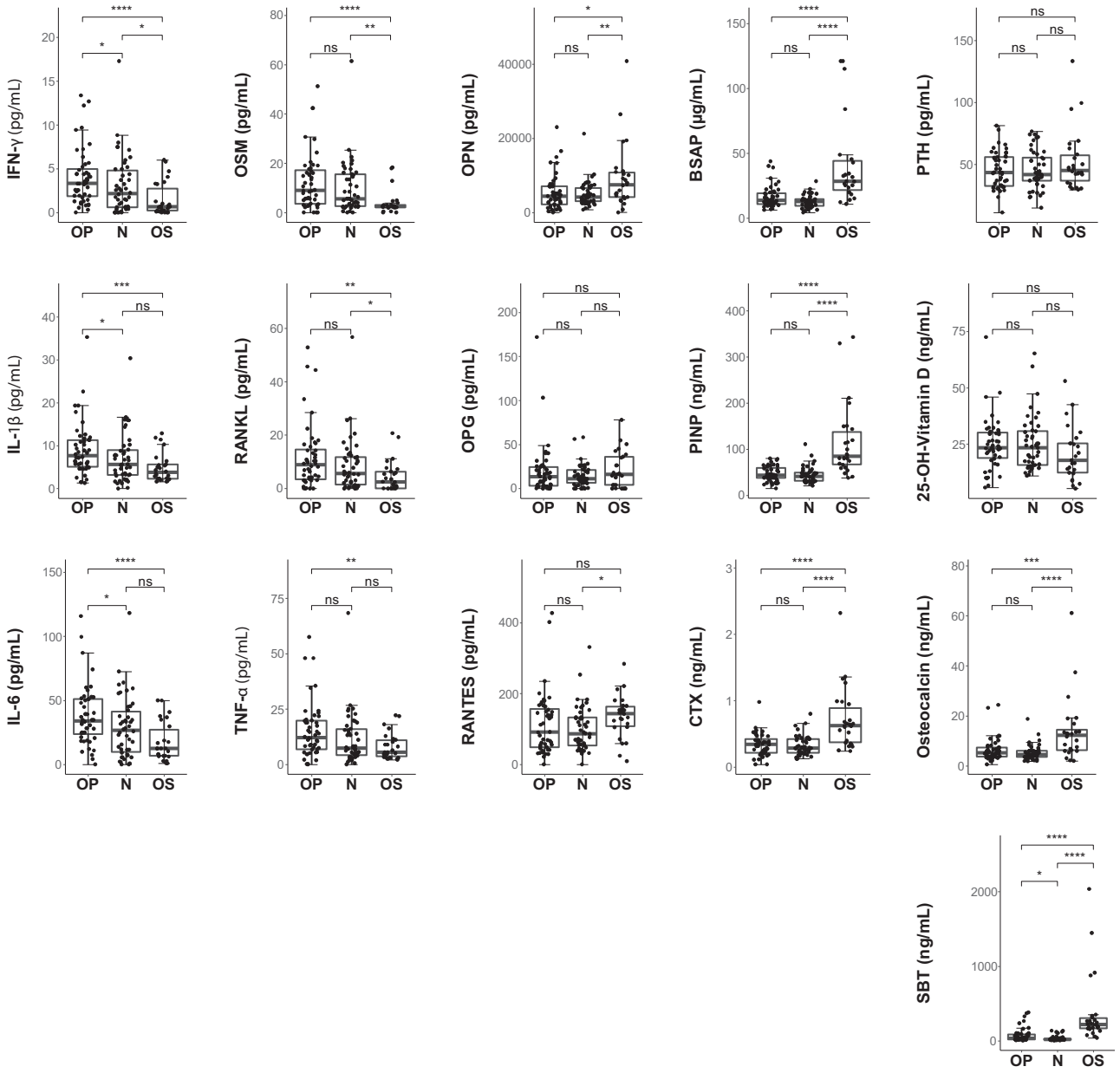

FIGURE 2. Cytokine and bone marker biochemical profile in patients with SM presenting with normal bone density (N), bone mass loss (OP), and diffuse bone sclerosis (OS). *ns*, Nonsignificant; *OP*, osteoporosis/severe osteopenia; *OS*, diffuse bone sclerosis. $*P < .05$; $**P < .01$; $***P < .001$; $****P < .0001$.

for continuous and categorical variables, respectively. The relationship between the levels of different cytokines and the T-score for the lumbar spine and the femoral neck was determined using the Spearman correlation test.

Clustering tendency was assessed by Hopkins statistics, and stability tests were performed to identify the optimal number of clusters and clustering methods. A hierarchical clustering model ($k = 4$) with a corresponding dendrogram and heatmap was built using the Euclidean distances between specific patient values and the mean value for each variable in the "no bone disease" group, based on those variables that either correlated with the lumbar spine and/or the femoral neck T-scores for BMD (Figure 1) or showed significantly different values among the distinct bone disease patient groups (Figure 2). Comparisons between clusters were performed using the χ^2 and Kruskal-Wallis tests, with the *post hoc* Bonferroni adjustment for pairwise comparisons.

For all statistical analyses, the SPSS for Windows (version 26.0, IBM Corporation, Armonk, NY) and R-Studio (version 1.3.959, RStudio, PBC, Boston, Mass) software packages were used.

RESULTS

Demographic and clinical features of patients with SM

Among the 120 patients, 58 (48%) were males, with a median age of 46 years (interquartile range, 36-55 years).

TABLE I. Demographic, clinical, and molecular features of patients with SM presenting without bone disease vs bone mass loss and/or diffuse bone sclerosis (n = 120)

Characteristic	No bone disease (n = 46)	Bone mass loss (n = 47)	Diffuse bone sclerosis (n = 27)	*P*
Sex: male	24 (52)	22 (46)	12 (46)	.79
Age (y)*	44 (18 to 74)	47 (25 to 77)	52 (21 to 74)	.12
Diagnosis				
BMM	21 (46)	19 (40)	0 (0)	**<.001**
ISM	21 (48)	22 (47)	17 (63)	.36
SSM	0 (0)	3 (6)	5 (19)	**.005**
SM-AHN	2 (4)	1 (2)	0 (0)	.62
ASM	1 (2)	2 (4)	5 (19)	**.045**
Clinical manifestations				
Skin lesions	23 (50)	24 (51)	20 (73)	.10
Anaphylaxis	30 (67)	25 (52)	8 (30)	**.01**
Associated diseases				
Chronic infections	0 (0)	2 (4)	0 (0)	.35
Autoimmune diseases	4 (9)	1 (2)	0 (0)	.18
Allergic diseases	30 (65)	27 (57)	8 (30)	**.01**
Drug hypersensitivity	9 (20)	9 (19)	8 (30)	.51
HVA	12 (26)	12 (26)	1 (4)	**.03**
Food allergy	7 (15)	8 (17)	1 (4)	.24
Allergic rhinitis	7 (15)	5 (11)	0 (0)	.09
Asthma	2 (4)	1 (2)	1 (4)	.84
Atopic dermatitis	1 (2)	1 (2)	0 (0)	>0.99
Multilineage *KIT*^{D816V}	6 (13)	11 (23)	22 (82)	**<.001**
BMD				
LS T score*	0 (−1.4 to 2.3)	−2.5 (−4.4 to 1.5)	2.4 (−1.1 to 6.7)	**<.001**
FN T score*	0.2 (−0.9 to 2.3)	−1.7 (−4.4 to 0.7)	3.2 (−0.5 to 6.0)	**<.001**

FN, Femoral neck; *HVA*, Hymenoptera venom allergy; *LS*, lumbar spine; *SSM*, smoldering SM; *SM-AHN*, SM associated with another hematological neoplasm.
Results expressed as number of patients from all patients in the group and percentage between parentheses (rounded to units).
Significant differences among groups are highlighted in bold.
*Results expressed as median value and range between parentheses.

According to the diagnostic subtype of mastocytosis, 40 (33%) patients had BMM, 61 (51%) ISM, 8 (7%) smoldering SM, 3 (3%) SM associated with another hematological (ie, myeloid) neoplasm, and 8 (7%) had ASM (see Tables E1 and E2 in this article's Online Repository at www.jaci-inpractice.org). Skin lesions were present in 67 (58%) patients, and 63 (53%) patients had had anaphylaxis (Table E1). Regarding the prevalence of potential causes for altered cytokine production and levels in plasma, other than SM, 2 (2%) patients had chronic infections, 5 (4%) had autoimmune diseases, 65 (54%) had atopy, and 65 (54%) had allergic diseases — 25 (21%) had Hymenoptera venom allergy, 26 (22%) drug hypersensitivity, 12 (10%) allergic rhinitis, 4 (3%) asthma, and 2 (2%) atopic dermatitis (Tables E1 and E2).

Overall, 47 (39%) patients had significant bone mass loss, 27 (23%) displayed diffuse bone sclerosis, and 46 (38%) patients showed no evidence of bone disease (Table E1). Of note, anaphylaxis ($P = .004$), atopy ($P = .004$), and Hymenoptera venom allergy ($P = .03$) were all significantly less frequent among patients who had diffuse bone sclerosis, whereas there were no significant differences across the 3 bone disease groups for the remaining variables (Table I). In turn, the presence of multilineage *KIT*^{D816V} was significantly more frequent among patients presenting with bone disease, particularly among those who had diffuse bone sclerosis ($P < .01$) (Table I).

Association between serum/plasma biomarkers in SM and the features of the disease

Overall, there were several differences for some of the biomarkers investigated among SM patients with distinct disease subtypes. Thus, IFN-γ levels were lower in patients with BMM versus ISM patients ($P = .028$), whereas BSAP levels were higher in ASM patients and smoldering SM as compared with those with BMM ($P < .01$ and $P < .01$, respectively) and ISM ($P = .024$ and $P < .01$, respectively). Likewise, PINP levels were higher in ASM patients as compared with those with BMM ($P = .027$) and ISM ($P < .01$), CTX levels were higher in ASM patients versus ISM ($P = .015$), and sBT was higher in ASM versus BMM ($P < .01$) and ISM ($P < .01$) (see Figure E1 in this article's Online Repository at www.jaci-inpractice.org). Except for osteocalcin, which was higher in female SM patients ($P = .01$), all other investigated biomarkers showed similar levels in male versus female patients (Table E1).

From a clinical point of view, SM patients who had anaphylaxis showed higher CTX ($P = .03$) and lower sBT ($P < .001$) levels (see Table E3 in this article's Online Repository at www.jaci-inpractice.org). Moreover, those who had no skin lesions showed higher sBT ($P < .001$), IFN-γ ($P = .04$), and IL-6 ($P = .02$) levels and lower osteocalcin values ($P = .006$) (Table E3). In turn, patients with allergic diseases displayed lower sBT values ($P = .002$) and BSAP ($P = .02$), and a trend toward lower PINP

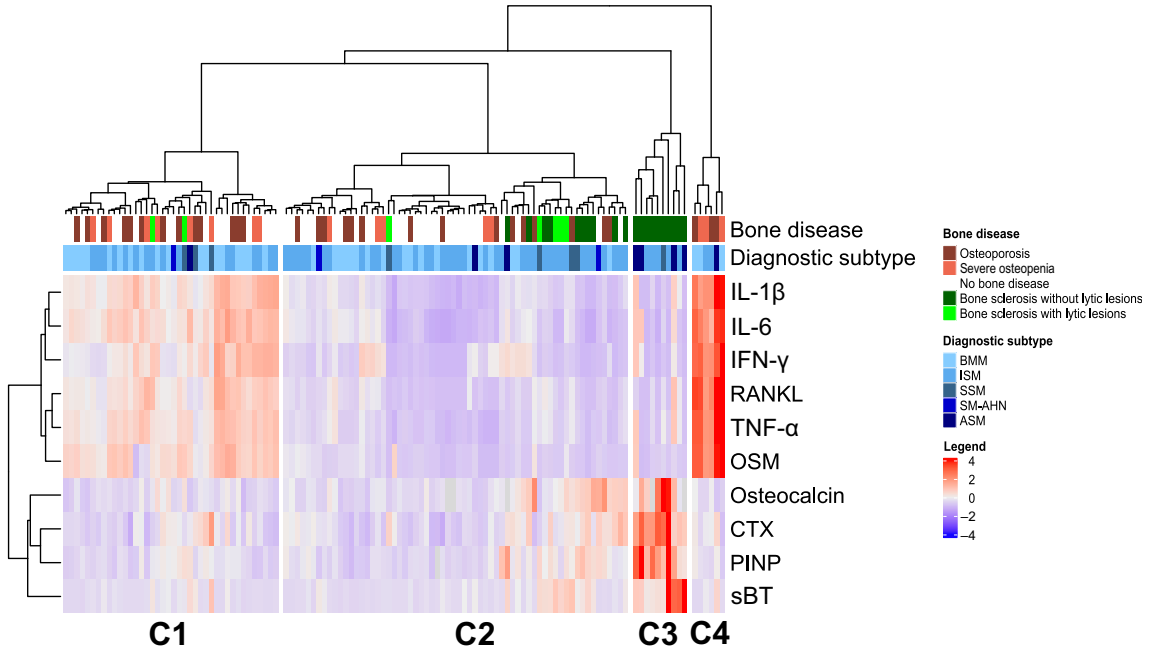

FIGURE 3. Two-dimensional heatmap representation of unsupervised hierarchical clustering analysis of patients with SM according to their cytokine/bone remodeling marker plasma/serum profiles. The bone disease status as well as the diagnostic subtype of SM is indicated for each individual patient in the two upper lines bellow the dendrogram. The color scale in the remaining lines refers to the abundance of each marker, denoted as squared Euclidean distance to the mean value of the marker in the "No bone disease" group, where red indicates a higher and blue a lower abundance than that observed in average among patients with SM who had no bone disease. *SSM*, Smoldering SM; *SM-AHN*, SM associated with another hematological neoplasm.

($P = .08$) (Table E3). This pattern was replicated among patients with Hymenoptera venom allergy, but not in patients with drug hypersensitivity among whom sBT levels were significantly higher ($P = .01$) (Table E3). In contrast, no significantly different biomarker serum/plasma levels were found between patients with and without chronic infections, or autoimmune diseases (Table E3).

Serum/plasma biomarkers and bone disease in SM

Interestingly, significant direct correlations were found between the lumbar spine bone density T-score and PINP ($r = 0.33$, $P < .001$), CTX ($r = 0.32$, $P < .001$), sBT ($r = 0.29$, $P = .001$), and osteocalcin ($r = 0.2$, $P = .03$) levels in serum/plasma, whereas inverse correlations were observed with IFN-γ ($r = -0.34$, $P < .001$), IL-6 ($r = -0.27$, $P = .003$), OSM ($r = -0.28$, $P = .002$), RANKL ($r = -0.27$, $P = .003$), IL-1β ($r = -0.26$, $P = .006$), TNF-α (r = −0.24, $P = .009$), and vitamin D ($r = -0.19$, $P = .049$) levels, respectively (Figure 1, *A*). In turn, those biomarkers that were directly correlated with the femoral neck bone density T-score included PINP ($r = 0.43$, $P < .0001$), sBT ($r = 0.36$, $P < .001$), CTX ($r = 0.35$, $P < .001$), osteocalcin ($r = 0.27$, $P = .004$), and BSAP ($r = 0.26$, $P = .006$). Conversely, IFN-γ ($r = -0.35$, $P < .001$), IL-6 ($r = -0.3$, $P = .001$), OSM ($r = -0.31$, $P < .001$), RANKL ($r = -0.3$, $P = .001$), IL-1β ($r = -0.27$, $P = .003$), TNF-α ($r = -0.24$, $P = .009$), and vitamin D ($r = -0.21$, $P = 0.03$) levels inversely correlated with the femoral neck bone density T-score (Figure 1, *B*).

Based on of these findings, we subsequently investigated the overall biomarker profile associated with patients with SM presenting with bone density loss and/or bone sclerosis, compared with those without bone alterations. Our results showed that patients with bone density loss had significantly higher levels of sBT ($P = .01$), IFN-γ ($P = .05$), IL-1β ($P = .05$), and IL-6 ($P = .05$), with a trend toward higher levels of BSAP ($P = .06$), TNF-α ($P = .07$), and OSM ($P = .08$) in serum/plasma, but similar plasma levels of CTX, osteocalcin, PINP, PTH, vitamin D, OPG, or RANKL (Figure 2). Within the bone mass loss group, there were no significant differences in the plasma/serum levels of any of the studied cytokines/bone remodeling markers between those patients presenting with severe osteopenia and those who had osteoporosis (see Figure E2 in this article's Online Repository at www.jaci-inpractice.org). In contrast, patients with diffuse bone sclerosis had significantly higher levels of sBT ($P < .0001$), CTX ($P < .001$), PINP ($P < .0001$), osteocalcin ($P < .0001$), BSAP ($P < .0001$), OPN ($P < .01$), and RANTES ($P = .01$), together with lower levels of IFN-γ ($P = .03$), OSM ($P < .01$), and RANKL ($P = .04$), and a trend toward lower IL-6 levels ($P = .06$), but similar vitamin D, PTH, IL-1β, OPG, or TNF-α plasma levels (Figure 2). Within the diffuse bone sclerosis group, those patients presenting without lytic bone lesions had higher levels of CTX ($P < .001$), PINP ($P = .01$), and osteocalcin ($P = .01$) compared with cases who displayed lytic bone lesions, whereas no significant differences were found for the levels of the remaining biomarkers between these two groups (see Figure E3 in this article's Online Repository at www.jaci-inpractice.org).

Unsupervised hierarchical clustering of patients with SM based on the serum/plasma levels of cytokine and bone

remodeling markers and sBT showed the presence of four different clusters. Cluster (C)1 included 40 patients (33%) with lower (below-average) levels of sBT and bone remodeling markers and higher (moderately above-average) levels of cytokines. In turn, C2 included 64 patients (53%) presenting with heterogeneous levels of sBT and bone remodeling markers and mostly moderately lower cytokine levels. C3 consisted of 10 patients (8%) presenting moderately to highly increased levels of sBT and increased bone remodeling markers, but moderately lower cytokine levels, whereas C4 consisted of only 6 patients (5%) with average levels of sBT and bone remodeling markers, associated with higher amounts of cytokines in plasma (Figure 3). Overall, bone mass loss was significantly more frequent in C4 (100%) than in C3 (0%; $P = .003$) and also in C2 (34%; $P = .03$) patients, whereas diffuse bone sclerosis (without lytic lesions) was almost exclusively found among C3 cases (100% vs 0% in C1, 16% in C2, and 0% in C4; $P = .02$) (Table II). Overall, this was associated with higher median (range) lumbar spine and femoral neck BMD T-scores of 2.4 (1.3-3.6) and 3.1 (2.3-4.1) in C3 patients versus −2.7 (−3.3 to −1.2; $P < .0001$) and −1.9 (−3.6 to −1.1; $P < .0001$) in C4, −0.5 (−4.4 to 6.7; $P < .0001$) and −0.1 (−3.3 to 6; $P = .003$) in C2, and −1 (−3.3 to 2.6; $P < .0001$) and −0.6 (−3.3 to 3.1; $P < .0001$) in C1, respectively (Table II). In addition, ASM was significantly more frequent among C3 cases (40%) than among C2 (3%; $P = .005$) and C1 (3%; $P = .02$) patients (Table II). In line with these later findings, multilineage involvement by KIT^{D816V} was also significantly more frequent among C3 (100%) than among C1 (28%; $P = .009$), C2 (27%; $P < .001$) and C4 (17%; $P = .02$) patients, in the absence of differences among the 4 clusters of patients with SM regarding other disease features (Table II; see Table E4 in this article's Online Repository at www.jaci-inpractice.org).

DISCUSSION

Bone disease is frequent across all diagnostic subtypes of mastocytosis,[2,3] and severe osteoporosis with pathological fractures is currently considered a C finding in both the WHO[23] and ICC[25] classifications of mastocytosis. Several MC mediators have been related to the development of bone disease in mastocytosis. Among other mechanisms, these include the resorptive effect of metalloproteases whose production is induced and/or enhanced by tryptase,[11-14] the promotion of osteoclastogenesis, and/or osteoclastic function by cytokines such as TNF-α, IL-1β, and IL-6, and the activation of the RANKL-induced pathway by histamine, prostaglandins,[28] platelet-activating factor,[28] leukotrienes,[29,30] heparin, and tryptase.[31] In contrast, the mechanisms leading to diffuse bone sclerosis, a much less frequent manifestation of bone disease in SM, remain largely unknown. Here, we investigated the potential association between serum/plasma levels of several relevant cytokines and bone remodeling markers and both types of bone disease in SM. Overall, our results showed that patients with SM cluster into 4 different groups, according to their cytokine/bone remodeling marker profiles. The first cluster consisted of a relatively homogeneous group of patients enriched in ASM cases with higher levels of bone remodeling markers and lower cytokine levels (TNF-α, RANKL, OSM, IFN-γ, IL-1β, and IL-6) in serum/plasma who systematically present diffuse bone sclerosis without lytic lesions, in association with multilineage involvement by the KIT^{D816V} mutation. The second group included patients with SM with very high cytokine levels but average levels of bone remodeling markers who only presented with osteoporosis. In turn, the third group of patients presented with normal to low BMD characterized by moderately high levels of cytokines and relatively low levels of bone remodeling markers. The fourth group included patients with SM presenting with heterogeneous (low to high) levels of bone remodeling markers and low cytokine levels associated with variable BMD T-score.

At present, it is well established that bone turnover is driven by RANKL produced by osteocytes, which provides a stimulatory signal that promotes the generation, differentiation, activation, and survival of osteoclasts.[15] In turn, bone resorption driven by osteoclasts is inhibited by OPG, which is also produced by osteoblasts to antagonize RANKL.[15] While high levels of RANKL are frequently observed in individuals presenting with osteoporosis,[32] low levels of RANKL have been associated with increased bone density in humans.[33] In mastocytosis, high levels of RANKL have been associated with bone loss,[17] whereas evidence of the effect of RANKL on increased bone density is still lacking. In turn, OPG has been shown to be increased in mastocytosis, particularly in advanced forms of the disease,[34] in line with our findings. However, no correlation was found between OPG levels and (lumbar spine/femoral neck) BMD or bone disease. RANKL was not elevated in our patients with SM presenting with bone loss, despite being inversely correlated with both the lumbar spine and femoral neck BMD. Interestingly, RANKL levels were significantly lower among patients with SM who had diffuse bone sclerosis, suggesting a compromised osteoclastic function in these cases.

Classical bone resorption—associated inflammatory cytokines such as IL-6, IL-1β, and TNF-α upregulate the osteoclastic function and inhibit osteoblasts, through different mechanisms.[35] IL-6 binds to its receptor on osteoclastic precursors where it induces their differentiation, and on osteoblasts where it promotes the activation of the Janus kinase/signal transducer and activator of transcription (JAK/STAT) 3 pathway with the release of PTH-related protein, RANKL, and IL-1, all of which upregulate the differentiation and function of osteoclasts.[36,37] In addition, IL-6 enhances bone resorption through inhibition of the differentiation of osteoblastic precursors via downregulation of the expression of the alkaline phosphatase, *Runx2*, and osteocalcin genes.[37] Moreover, IL-1β promotes the differentiation and activation of osteoclasts by upregulating the expression of RANKL and activation of the p38 mitogen-activated protein kinase pathway.[38] Similarly, TNF-α promotes the genesis, differentiation, and function of osteoclasts through several mechanisms that include the activation of the nuclear factor kappa B and AP-1 pathways, expression of RANK, and upregulation of the production of other proresorptive cytokines.[38] Although IL-6 seems to have an established role in osteoporosis secondary to mastocytosis,[17,39] evidence on the relevance of IL-1β and TNF-α in bone mass loss is scarce and limited to preclinical studies and findings in other inflammatory diseases, such as rheumatoid arthritis[7,40]; at the same time, their role in diffuse bone sclerosis remains to be determined. Here, we provide new evidence that suggests that among patients with SM, IL-6, IL-1β, and TNF-α are particularly increased in patients suffering from significant bone mass loss (both severe osteopenia and osteoporosis), in line with previous observations.[16] In turn, lower IL-6 levels were found in diffuse bone sclerosis, its levels being inversely

TABLE II. Demographic, clinical, and molecular features of patients with SM included in different clusters defined on the basis of sBT and plasma/serum levels of other cytokine and bone remodeling markers

Characteristic	C1 (n = 40)	C2 (n = 64)	C3 (n = 10)	C4 (n = 6)	*P*
Sex: male	20 (50)	32 (50)	4 (40)	2 (33)	.85
Age (y)*	48 (18 to 75)	43 (21 to 74)	51 (34 to 63)	43 (30 to 77)	.44
Bone disease					
No bone disease	17 (43)	29 (45)	0 (0)	0 (0)	**.005**
Bone mass loss	21 (53)	22 (34)	0 (0)	6 (100)	**<.001**
Severe osteopenia	8 (20)	6 (9)	0 (0)	2 (33)	.11
Osteoporosis	13 (33)	16 (25)	0 (0)	4 (67)	**.02**
Diffuse bone sclerosis	2 (5)	14 (22)	10 (100)	0 (0)	**<.001**
Without lytic lesions	0 (0)	10 (16)	10 (100)	0 (0)	**.02**
With lytic lesions	2 (5)	4 (6)	0 (0)	0 (0)	.89
Diagnosis					
BMM	19 (48)	18 (28)	0 (0)	3 (50)	**.01**
ISM	16 (40)	38 (59)	5 (50)	2 (33)	.20
SSM	3 (8)	4 (6)	1 (10)	0 (0)	.91
SM-AHN	2 (5)	3 (5)	0 (0)	0 (0)	>0.99
ASM	1 (3)	2 (3)	4 (40)	1 (17)	**.001**
Clinical manifestations					
Skin lesions	18 (45)	39 (61)	7 (70)	3 (50)	.33
Anaphylaxis	21 (53)	36 (56)	4 (40)	2 (33)	.62
Associated diseases					
Chronic infections	2 (5)	0 (0)	0 (0)	0 (0)	.36
Autoimmune diseases	2 (5)	3 (5)	0 (0)	0 (0)	>0.99
Allergic diseases	22 (55)	36 (56)	4 (40)	3 (50)	.83
Drug hypersensitivity	7 (18)	13 (20)	5 (50)	1 (17)	.17
HVA	10 (25)	13 (20)	0 (0)	2 (33)	.25
Food allergy	6 (15)	10 (16)	0 (0)	0 (0)	.59
Allergic rhinitis	5 (13)	7 (11)	0 (0)	0 (0)	.82
Asthma	2 (5)	1 (2)	1 (10)	0 (0)	.35
Atopic dermatitis	0 (0)	1 (2)	0 (0)	1 (17)	.10
Multilineage *KIT*^{D816V}	11 (28)	17 (27)	10 (100)	1 (17)	**<.001**
BMD					
LS T score*	−1 (−3.3 to 2.6)	−0.5 (−4.4 to 6.7)	2.4 (1.3 to 3.6)	−2.7 (−3.3 to −1.2)	**<.001**
FN T score*	−0.6 (−3.3 to 3.1)	−0.1 (−3.3 to 6)	3.1 (2.3 to 4.1)	−1.9 (−3.6 to −1.1)	**<.001**

FN, Femoral neck; *HVA*, Hymenoptera venom allergy; *LS*, lumbar spine; *SSM*, smoldering SM; *SM-AHN*, SM associated with another hematological neoplasm.
Results expressed as number of patients from all patients in the group and percentage between parentheses (rounded to units).
Significant differences among groups are highlighted in bold.
*Results expressed as median value and range between parentheses.

correlated with both the lumbar spine and the femoral neck BMD. Altogether, these findings support a major role for inflammation in the pathogenesis of bone loss in mastocytosis, whereas a more immunosuppressed milieu may be associated with diffuse bone sclerosis. In addition, our results show for the first time that previously described differences among distinct diagnostic subtypes of mastocytosis for IL-6 and IL-1β[41] tend to reside in the setting of a bone disease—controlled-study population, and might be confounded by the presence versus absence of bone disease.

The role of cytokines other than IL-1 and IL-6 in bone remodeling is less well established. Despite this, previous studies suggest that IFN-γ might exert a dose-dependent effect on osteoclasts.[42] In fact, low levels of IFN-γ might exert an effect on BM osteoclast precursors through TNF Receptor Associated Factor (TRAF) 6-mediated degradation of RANKL, or Nuclear factor of activated T-cells, cytoplasmic (NFATc) 1, promoting their apoptosis by a Fas-Fas ligand interaction.[42] However, at higher doses, IFN-γ may induce the terminal maturation of osteoclasts.[43] Of note, recombinant IFN-γ-1b is among the therapeutic armamentarium recommended for the treatment of osteopetrosis, a group of diseases characterized by impaired osteoclastic functionality.[44] In line with these findings, here we found IFN-γ levels to be inversely correlated with BMD: higher in osteoporosis/severe osteopenia, while decreased in diffuse bone sclerosis. These results suggest a positive effect of IFN-γ on osteoclastic function in mastocytosis, paving the way for future research and (potentially also) for new clinical trials exploring the use of recombinant IFN-γ-1b in diffuse bone sclerosis secondary to SM. OSM is another member of the IL-6 family that stimulates the production of receptor activator of RANKL on osteoblasts, thereby driving the formation of osteoclasts, particularly in pathological conditions.[45] Previous studies have shown that OSM is increased in patients with

mastocytosis,[16] but its effects on mastocytosis-related bone disease remain unknown. Our results show that in SM, OSM levels are inversely correlated with the lumbar spine and the femoral neck BMD. At the same time, they are significantly lower among patients with diffuse bone sclerosis, whereas they tend to be upregulated in osteoporosis, in line with previously reported data. RANTES is a chemokine that may be released by MCs, osteoclasts, and T cells, which is known to chemoattract osteoblasts.[16] Here, we showed for the first time that RANTES levels are significantly upregulated in patients with diffuse bone sclerosis, in the absence of a significant correlation with bone mass loss, supporting a limited role for RANTES on osteoblasts in the pathogenesis of diffuse bone sclerosis in SM.

Several bone remodeling markers other than the aforementioned cytokines have been proven to be reliable biomarkers of bone turnover. Thus, some bone formation markers such as BSAP and osteocalcin have been associated with the osteoblastic function, whereas other markers such as PINP (which reflects the synthesis of new collagen)[46] and CTX (a collagen fragment of the α-1 peptide from the C-telopeptide region released during degradation of type II collagen) are regarded as bone resorption–associated markers.[46] Nonetheless, elevated levels of CTX have been found in patients with osteosclerosis[47,48] and osteoblastic tumors,[49] and in a small series of patients with mastocytosis with diffuse bone sclerosis.[50] Overall, our data showed higher CTX levels in patients with SM presenting with diffuse bone sclerosis, its levels directly correlating with BMD. This emerges as a relevant finding because other osteoclastic bone resorption markers such as RANKL are diminished in diffuse bone sclerosis, highlighting the potential use of CTX as a biomarker for excessive osteoblastic function. As might had been expected, bone formation markers such as osteocalcin and PINP were found to be increased in our cohort among those patients who had diffuse bone sclerosis, with a significant correlation with the lumbar spine and femoral neck BMD. OPN is a glycoprotein released by osteocytes, osteoblasts, and MCs that may be involved in bone remodeling, but whose role as a bone marker remains controversial.[51] Thus, although some studies showed an inverse correlation between OPN levels and BMD in osteoporosis,[52] its role in osteosclerosis remains to be established.[53] Similarly, the expression of the *SPP1* gene, which codes for OPN, has been reported to be increased in ASM,[16] a specific subtype of SM in which bone sclerosis is more frequent, as also confirmed here.

Tryptase is a serine protease produced and released by MCs and (to a much lesser extent) by basophils.[17] A limited amount of pro-α-tryptase is constitutively secreted by unstimulated MCs and comprises the vast majority of sBT,[54] whereas mature β-tryptase tetramers are present in the cytoplasmic granules of MCs, being released on MC degranulation.[55] Besides being a biomarker for MC activation and MC burden, tryptase might contribute to paraneoplastic manifestations of SM (eg, connective tissue degradation) through direct activation of several members of the matrix metalloproteases pathway (mostly, β-tryptase),[11-14] proliferation and chemotaxis of fibroblasts, proliferation of epithelial cells and smooth muscle cells, and promotion of the synthesis of collagen (mostly by α-tryptase).[56-59] In mastocytosis, sBT levels have also been shown to correlate with the BM MC and *KIT*^{D816V} allele burden.[60] Regarding bone disease in SM, increased sBT levels have been associated with both bone sclerosis (due to unknown mechanisms)[8,18,19] and osteoporosis (it has been hypothesized that tryptase could induce the production of OPG).[4,17,61] Here, we showed that sBT values are higher in patients presenting with bone loss, compared with patients who had no signs of bone disease. Nonetheless, sBT levels tended to be even higher in patients with diffuse bone sclerosis, which translated into a significant correlation between sBT levels and both the lumbar spine and the femoral neck BMD. These findings are in line with previous data showing a correlation between the MC burden and the development of BM fibrosis in SM, as well as in other myeloproliferative neoplasms.[62-64] Whether tryptase should be considered as a direct stimulus for BM fibrosis and bone sclerosis, or whether it just reflects the MC burden associated with bone sclerosis, deserves further investigation. Based on all the above, it could be hypothesized that bone sclerosis might result from the proliferative effect of constitutively released α-tryptase occurring in patients presenting with an increased burden of immunophenotypically (more) immature BM MC (frequently, presenting with multilineage *KIT*^{D816V}[65]). In turn, increased bone remodeling and the resulting bone mass loss might be associated with the frequent release of proteolytically active β-tryptase, occurring in patients presenting with an activated BM MC immunophenotype (usually BMM/ISM[66]).

Consensus recommendations for the general population[67] in which a pharmacological intervention is recommended for patients presenting with (1) a BMD T-score of −2.5 or below by dual-energy X-ray absorptiometry; (2) a T-score of between −1.0 and −2.5 accompanied by an osteoporosis-related fracture (FRAX) risk of 3% or more for femoral neck or total hip fracture; or (3) a risk of 20% or more for a 10-year major osteoporosis-related (ie, clinical vertebral, hip, forearm, or proximal humerus) fracture regardless of BMD; or (4) a T-score of between −1.0 and −2.5 and a previous history of fracture of the proximal humerus, pelvis, or distal forearm. In patients with SM, antiresorptive therapy (sequential administration of bisphosphonates, RANKL inhibitor, and low-dose IFN-α) is recommended for those patients presenting with a BMD T-score of less than 2, regardless of other factors.[68] However, the latter recommendations are based on clinical experience and a perception of high risk for pathologic/trauma-related bone fractures in patients with SM, but they lack validation studies and cost-benefit analyses. Overall, our results suggest that SM presenting with severe osteopenia and osteoporosis show similar features regarding the cytokine and bone remodeling marker milieu, thus confirming the plausibility of early pharmacologic intervention for the treatment of bone mass loss in patients with SM.

CONCLUSION

Here, we demonstrated for the first time that patients with SM may display distinct profiles in plasma/serum cytokines and other bone remodeling biomarkers that are closely and distinctly associated with bone mass loss and diffuse bone sclerosis as reflected by a typical proinflammatory/osteoclastogenic cytokine profile in patients presenting with bone mass loss, and an increase in plasma/serum bone turnover biomarkers and an immunosuppressive milieu with lower IFN-γ levels in diffuse bone sclerosis cases. Further longitudinal long-term studies in large cohorts of patients with SM and age and sex–matched controls are needed for a more in-depth understanding of the

role of these biomarkers in the development of bone disease in SM and the underlying mechanisms involved.

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