

Clinical, immunophenotypic, and molecular characteristics of well-differentiated systemic mastocytosis

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Background: Well-differentiated systemic mastocytosis (WDSM) is a rare variant of systemic mastocytosis (SM) characterized by bone marrow (BM) infiltration by mature-appearing mast cells (MCs) often lacking exon 17 *KIT* mutations. Because of its rarity, the clinical and biological features of WDSM remain poorly defined.

Objective: We sought to determine the clinical, biological, and molecular features of a cohort of 33 patients with mastocytosis in the skin in association with BM infiltration by well-differentiated MCs and to establish potential diagnostic criteria for WDSM.

Methods: Thirty-three patients with mastocytosis in the skin plus BM aggregates of round, fully granulated MCs lacking strong CD25 and CD2 expression in association with clonal MC features were studied.

Results: Our cohort of patients showed female predominance (female/male ratio, 4:1) and childhood onset of the disease (91%) with frequent familial aggregation (39%). Skin involvement was heterogeneous, including maculopapular (82%), nodular (6%), and diffuse cutaneous (12%) mastocytosis. *KIT* mutations were detected in only 10 (30%) of 33 patients, including the *KIT* D816V (n = 5), K509I (n = 3), N819Y (n = 1), and I817V (n = 1) mutations. BM MCs displayed a unique immunophenotypic pattern consisting of increased light scatter features, overexpression of cytoplasmic carboxypeptidase, and aberrant expression of CD30, together

with absent (79%) or low (21%) positivity for CD25, CD2, or both. Despite only 9 (27%) of 33 patients fulfilling the World Health Organization criteria for SM, our findings allowed us to establish the systemic nature of the disease, which fit with the definition of WDSM.

Conclusions: WDSM represents a rare clinically and molecularly heterogeneous variant of SM that requires unique diagnostic criteria to avoid a misdiagnosis of cutaneous mastocytosis per current World Health Organization criteria. (J Allergy Clin Immunol 2015;■■■■:■■■-■■■.)

Key words: Mast cell, mastocytosis, well differentiated, KIT, imatinib

Mastocytosis consists of a heterogeneous group of disorders characterized by the accumulation of clonal mast cells (MCs) in different organs and tissues often associated with MC mediator release symptoms. Seven variants of the disease plus a provisional subvariant are currently recognized by the World Health Organization (WHO) 2008 classification of mastocytosis¹: cutaneous mastocytosis (CM), indolent systemic mastocytosis (ISM), aggressive systemic mastocytosis (ASM), systemic mastocytosis with an associated clonal hematologic non-mast cell lineage disease (SM-AHNMD), mast cell leukemia (MCL), MC sarcoma, and extracutaneous mastocytoma, plus a provisional subvariant of ISM termed smoldering systemic mastocytosis (SSM).

In the WHO 2008 classification, systemic mastocytosis (SM) is defined by the presence of MC aggregates in the bone marrow (BM; major diagnostic criterion) in association with 1 or more minor criteria (morphologically atypical BM MCs, aberrant expression of CD25⁺ and/or CD2⁺, presence of *KIT* mutations at codon 816, and/or increased serum tryptase [sT] level >20 µg/L) or 3 or more of the above 4 minor criteria if MC aggregates are not detected.¹⁻³ In addition to SSM, recent reports have also identified a potential new subvariant of SM that has been termed well-differentiated systemic mastocytosis (WDSM). The few WDSM cases reported thus far have been shown to frequently have mature-appearing CD25^{-/low}/CD2^{-/low} BM MCs and to often lack on the D816V *KIT* mutation, therefore mimicking normal mature/differentiated MCs on both phenotypic and morphologic grounds.⁴⁻¹² This implies that 3 of the 4 minor WHO 2008 criteria for SM appear to be absent in this small subgroup of patients; in such a case, the presence of BM MC aggregates in association with increased sT levels would become a requirement for the diagnosis of SM per the WHO 2008 criteria among many patients with WDSM. However, preliminary results based on limited numbers of cases suggest that sT levels of less than 20 µg/L in the absence of other minor WHO 2008 diagnostic

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Abbreviations used

ASM:	Aggressive systemic mastocytosis
BM:	Bone marrow
CM:	Cutaneous mastocytosis
CPA:	Carboxypeptidase A
DCM:	Diffuse cutaneous mastocytosis
FACS:	Fluorescence-activated cell sorting
FSC:	Forward light scatter
GIST:	Gastrointestinal stromal tumor
HUMARA:	Human androgen receptor assay
ISM:	Indolent systemic mastocytosis
MC:	Mast cell
MCL:	Mast cell leukemia
MIS:	Mastocytosis in the skin
MPCM:	Maculopapular cutaneous mastocytosis
NM:	Nodular cutaneous mastocytosis
SM:	Systemic mastocytosis
SM-AHNMD:	Systemic mastocytosis associated with a clonal hematologic non-mast cell lineage disease
SSC:	Sideward light scatter
SSM:	Smoldering systemic mastocytosis
sT:	Serum tryptase
TKI:	Tyrosine kinase inhibitor
WDSM:	Well-differentiated systemic mastocytosis
WHO:	World Health Organization
XCIP:	X-chromosome inactivation pattern

criteria can occur frequently in this group of patients with SM, despite clear systemic disease involvement (eg, skin lesions and BM MC infiltration).^{7,9} Altogether, these findings point out the need for refined (eg, additional) diagnostic criteria for patients with WDSM. This becomes particularly relevant because of the low frequency of codon 816 *KIT* mutations reported in these patients,⁶ which would make them potential candidates for *KIT*-targeted tyrosine kinase inhibitor (TKI) therapy, as supported by the dramatic response to imatinib observed in a few case reports.^{4,10-12}

In this study we describe in detail the clinical, biological, histopathologic, cytomorphologic, immunophenotypic, and molecular characteristics of a group of patients with mastocytosis who had BM infiltration by clonal MCs displaying well-differentiated MC features similar to those found in patients with previously reported WDSM.

METHODS**Patient selection and evaluation**

Of 855 patients who underwent a complete BM study at the Spanish Network on Mastocytosis between January 1997 and December 2013 because of suspected SM, 33 (4%; 6 male and 27 female patients) were included in this study on the basis of the following criteria: (1) diagnosis of mastocytosis in the skin (MIS) confirmed by skin biopsy according to consensus criteria²; (2) demonstration of a homogeneous population of round and well-granulated CD25^{−/low} and CD2^{−/low} MCs in the BM, as assessed by means of cytomorphology and flow cytometry, respectively; and (3) evidence for BM clonal MC disease, as defined by the demonstration of aggregates of 15 or more MCs per cluster in BM sections or BM smears, mutations involving any exon of the *KIT* gene in purified BM MCs, and/or a clonal X-chromosome inactivation pattern (XCIP) in purified BM MCs from female patients, as assessed by using the human androgen receptor assay (HUMARA).^{13,14} All patients (or parents in the case of children) provided written informed consent to participate in the study, according to the Declaration of Helsinki. The study

was approved by the Institutional Ethics Committee of the Complejo Hospitalario de Toledo (Toledo, Spain).

Diagnostic clinical and laboratory work-up included evaluation and classification of skin lesions according to updated WHO criteria,¹⁵ as well as recording clinical symptoms related to MC mediator release (eg, pruritus, flushing, palpitations, headache, abdominal cramping, diarrhea, hypotension, and syncope). sT levels (UniCAP; Pharmacia, Uppsala, Sweden) were measured at the time of BM studies. Bone mass density and the presence of organomegaly were evaluated by using dual energy x-ray absorptiometry and abdominal ultrasonography, computerized tomography, or both, respectively.

Skin histopathology

Formalin-fixed, paraffin-embedded skin biopsy specimens were stained for hematoxylin and eosin, c-kit, and tryptase; in most cases, CD25 and CD30 were also evaluated. Diagnosis of MIS was based on either the presence of aggregates of more than 15 MCs per cluster or scattered MCs exceeding 20 cells per microscopic high-power field (×40 magnification), according to consensus criteria.² The pattern of dermal distribution of MCs and the presence of promastocytes and non-MC lineage cells (eg, lymphocytes, neutrophils, and eosinophils) were also recorded.

BM studies

Diagnostic BM aspiration was systematically performed in every patient, and biopsy samples were obtained only from adult patients according to previously proposed recommendations.^{2,16-19} BM aspirate smears were stained for toluidine blue and May-Grünwald-Giemsa for cytomorphologic evaluation, whereas BM sections were stained for hematoxylin and eosin, tryptase, c-kit, reticulin, CD25, and CD30. Multiparameter flow cytometric immunophenotypic studies were performed on whole BM aspirated samples by using 4- to 8-color staining, according to well-defined consensus procedures.^{16,17,19} Screening for the *KIT* D816V mutation and other exon 17 *KIT* mutations was performed in genomic DNA from a fluorescence-activated cell sorting (FACS)-purified populations of BM MCs, eosinophils, neutrophils, lymphocytes, and CD34⁺ hematopoietic progenitor cells, as described elsewhere.^{6,20} Mutations involving other exons of the *KIT* gene were also investigated in purified BM MCs from those patients who showed no exon 17 *KIT* mutations (see Table E1 in this article's Online Repository at www.jacionline.org). Among these latter patients, MC clonality was also determined in FACS-purified BM MCs from 14 women (11 of whom had BM aggregates consisting of >15 MCs, mutations involving codons other than codon 17 of the *KIT* gene, or both) by using HUMARA.^{13,14}

Statistical methods

Medians and ranges were calculated for continuous variables, and frequencies were reported for categorical variables. Statistical significance of differences observed between groups of patients was assessed by using the Mann-Whitney *U* and χ^2 tests for continuous and categorical variables, respectively. The Pearson correlation coefficient test was used for correlation studies between 2 variables. All statistical analyses were assessed for statistical significance at a threshold *P* value of less than .05 and performed with the SPSS 17.0 software package (SPSS, Chicago, Ill).

RESULTS**Clinical presentation**

Median age at diagnosis of the 33 patients studied was 27 years (range, 2-72 years). Despite this, all but 3 (91%) patients had a pediatric onset (<14 years old) of skin lesions, skin lesions arose within the first year of life in nearly half of such cases (median age at onset of skin lesions, 3 years; range, birth to 60 years; Table I), and first-degree familial aggregation was present in 13 (39%) patients (Table I). The great majority of patients (27/33 [82%])

TABLE I. Clinical characteristics of patients with WDSM (n = 33)

MC mediator release symptoms														
Patient ID	Sex	Age at diagnosis*	Age at onset*	Familial aggregation	Cutaneous involvement			MC mediator release symptoms						
					Type	Distribution	Extension†	Pruritus (grade%)	CV (grade%)	GI (grade%)	Anaphylaxis (frequency#)	Triggers of anaphylaxis	Organomegaly	BMD
1	F	26 y	4 y	Mother	MPCM	T+N	3	Yes (2)	Fl (2)	No	No	—	H	Normal
2	M	26 y	3 mo	No	MPCM	T+N+E	3	Yes (2)	Fl+P+S (4)	No	Yes (A)	NSAIDs	No	Normal
3	F	25 y	3 y	Brother	MPCM	T+N	2	No	Fl+P+D (2)	p (2)	No	—	No	Normal
4	F	26 y	4 y	No	MPCM	T+N	2	Yes (2)	Fl+P+D+S (4)	d,c (2)	Yes (A)	Idiopathic	No	Normal
5	F	44 y	41 y	No	MPCM	T	2	Yes (2)	No	No	No	—	No	Osteopenia
6	F	25 y	3 mo	No	MPCM	T+N+E	3	Yes (2)	No	d,c (2)	No	—	No	Normal
7	F	30 y	2 y	No	MPCM	T+N+E	2	Yes (2)	Fl (3)	No	No	—	No	Osteopenia
8	F	26 y	2 y	No	MPCM	T+N	2	Yes (2)	Fl+P+D (3)	d,p (2)	No	—	No	Normal
9	F	33 y	4 y	Son	MPCM	T+N	1	Yes (2)	Fl+P (3)	No	No	—	No	Not tested
10	F	29 y	1 y	Mother	MPCM	T+N	2	Yes (2)	No	No	No	—	No	Osteopenia
11	F	69 y	60 y	No	NM	E	1	No	Fl+P+Hyp+S (4)	No	Yes (B)	Idiopathic	No	Osteoporosis
12	F	24 y	1 y	No	MPCM	T+N	3	No	F (3)	No	No	—	H	Osteopenia
13	M	31 y	9 y	No	MPCM	T	1	Yes (2)	Fl+P (2)	No	No	—	No	Osteopenia
14	F	43 y	5 y	No	MPCM	T	1	Yes (2)	Fl+P+D (2)	No	Yes (A)	Idiopathic	No	Normal
15	F	24 y	6 y	No	MPCM	T+N	1	No	Fl (1)	No	No	—	No	Osteopenia
16	F	21 y	10 mo	No	MPCM	T+N	2	Yes (2)	Fl+P+D (2)	No	Yes (A)	Idiopathic	H	Normal
17	F	12 y	1 mo	No	MPCM‡	T+N+E	3	Yes (1)	Fl+S (2)	d (2)	Yes	Anesthesia	No	Osteopenia
18	F	21 y	14 y	No	MPCM	T+N	2	Yes (1)	Fl (1)	No	No	—	No	Normal
19	F	44 y	1 mo	Daughter	MPCM	T	1	Yes (1)	Fl (1)	No	No	—	No	Not tested
20	F	72 y	3 mo	No	MPCM§	T+N+E	4	Yes (3)	Fl+Hyp+S (4)	No	Yes (A)	Idiopathic	Sp	Osteoporosis
21	F	28 y	6 y	No	MPCM	T+N	1	No	Fl+P+D (1)	No	No	—	No	Osteoporosis
22	F	45 y	11 y	Daughter	MPCM	T	1	Yes (1)	Fl+P+D (1)	No	No	—	No	Normal
23	M	55 y	NB	Daughter (x2)	DCM	T+N+E	4	Yes (1)	No	d,p (1)	No	—	H	Normal
24	F	20 y	3 mo	Father, sister	DCM	T+N+E	4	Yes (2)	Fl (1)	d,p (1)	No	—	No	Normal
25	F	27 y	3 mo	Father, sister, son, daughter	DCM	T+N+E	4	Yes (2)	Fl (1)	d,c (2)	No	—	No	Normal
26	M	24 y	4 y	No	MPCM	T	1	Yes (1)	Fl+P (2)	No	No	—	No	Normal
27	F	18 y	3 y	Sister, brother	MPCM	T+N	2	No	No	No	No	—	No	Normal
28	F	22 y	5 y	No	MPCM	T+N	2	Yes (1)	Fl+P (1)	c (1)	No	—	No	Normal
29	F	67 y	5 y	Daughter	MPCM	T	1	Yes (1)	Fl (1)	No	No	—	No	Normal
30	F	40 y	NB	Daughter	MPCM	T+N	2	Yes (2)	Fl+D (2)	c,p (2)	Yes (A)	NSAIDs	No	Osteopenia
31	F	39 y	6 y	Daughter	MPCM	T+N	2	Yes (1)	Fl+P+D (2)	No	No	—	No	Normal
32	M	41 y	NB	No	DCM	T+N+E	4	Yes (1)	Fl (1)	No	No	—	No	Normal
33	M	2 y	NB	No	NM	T+N+E	4	Yes (3)	Fl+P+Hyp+S (4)	d,c (2)	Yes (C)	Idiopathic	H, Sp	Not tested
Total	F: 27 (82%)	27 y (2-72 y)	Childhood onset: 30 (91%)	13 (39%)	MPCM: 27 (82%)	T±N: 32 (97%)		27 (82%)	28 (85%)	11 (33%)	9 (27%)		6 (18%)	Bone mass loss: 11 (37%)

BMD, Bone mass density; c, abdominal cramping; CV, cardiovascular symptoms; D, dizziness; d, diarrhea; E, extremities; F, female; Fl, flushing; GI, gastrointestinal symptoms; H, hepatomegaly; Hyp, hypotension; M, male; N, neck; NB, newborn; NM, nodular mastocytosis; NSAID, nonsteroidal anti-inflammatory drug; P, palpitations; p, peptic symptoms; S, syncope; Sp, splenomegaly; T, trunk.

*In years, unless otherwise specified.

†Extension of cutaneous involvement was graded as follows: (1) skin lesions involving less than 5% of the body surface area, (2) skin lesions involving 5% to 25% of the body surface area, (3) skin lesions involving 25% to 50% of the body surface area, and (4) skin lesions involving greater than 50% of the body surface area.

‡The patient showed a gradual improvement in her skin lesions until they completely disappeared 3 years before the BM study.

§The patient had additional multiple mastocytomas located in her underarms and inner thighs, which emerged several months before she was referred for the BM study.

||Expressed in medians (ranges).

¶According to standardized consensus recommendations (see Valent et al² for more details): grade 1, mild, no therapy required; grade 2, moderate, kept under control with antimediator-type drugs; grade 3, severe, not sufficiently controlled with therapy; and grade 4, severe adverse event, requires emergency therapy and hospitalization.

#Frequency A, <1/y; frequency B, >1/y and <1/mo; frequency C, >1/mo.

had maculopapular cutaneous mastocytosis (MPCM), 4 (12%) patients had diffuse cutaneous mastocytosis (DCM), and 2 (6%) patients had nodular cutaneous mastocytosis (NM; Table I). MPCM skin lesions were small (usually <0.5 cm) brown to reddish macules and papules with variable degrees of extent (from isolated to multiple skin lesions), and they mainly involved the trunk and neck, whereas the extremities were often free of lesions (Table I). Compared with other body areas, MPCM lesions involving the neck were usually more elevated and showed a (micro)nodular macroscopic appearance (Fig 1, A). Patients with DCM presented with generalized thickening of the skin and exaggeration in skin folds, particularly in underarm skin, in the absence of individual hyperpigmented skin lesions

(Fig 1, B). The only 2 patients with NM were a 69-year-old woman with an adult-onset solitary mastocytoma of 8 × 5 cm in diameter located in her right arm that had been surgically removed before diagnosis and a 2-year-old child with neonatal multiple nodular lesions involving more than 90% of his body surface area (Fig 1, C). Of note, skin lesions at referral were observed in all but 1 patient, who showed gradual improvement of her skin lesions until they completely disappeared 3 years before the BM study.

The most frequent MC mediator release symptom was flushing (85% of patients), which presented as recurrent episodes usually accompanied by other vasodilatory symptoms (eg, palpitations, dizziness, headache, hypotension, or syncope), followed by

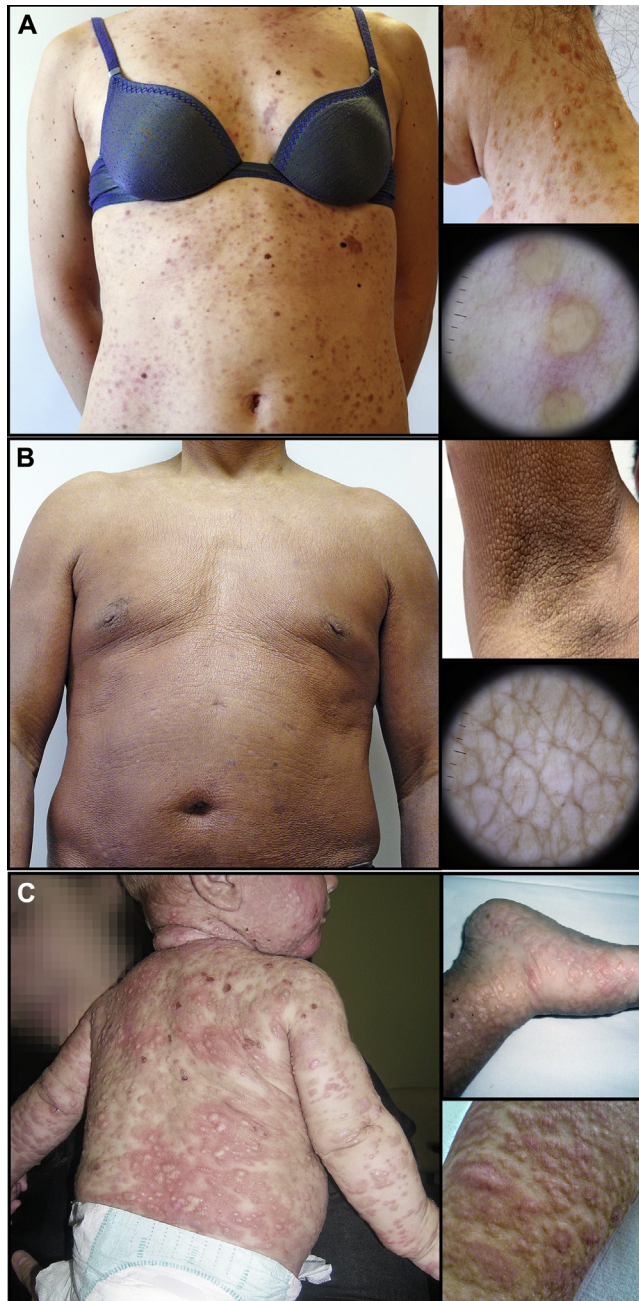


FIG 1. Different types of cutaneous involvement detected in patients with WDSM. **A**, MPCM in a 24-year-old woman (patient 2). Skin lesions in the neck mimicked micronodules and showed a yellow-orange blot dermoscopic pattern. **B**, DCM in a 55-year-old man (patient 23) who presented with generalized thickening of the skin with accentuation of folds that was particularly evident in the underarm skin. **C**, Extensive NM in a 2-year-old boy (patient 33) who had increased sT levels (121 $\mu\text{g/L}$) at diagnosis, probably reflecting the extensive cutaneous involvement coexisting with severe MC activation events.

pruritus (82%). Fourteen (42%) patients fulfilled the criteria for anaphylaxis (Table I). Overall, chronic gastrointestinal symptoms were observed in 33% of patients, and these included diarrhea (24%), abdominal cramping (21%), and dyspepsia (16%). Mild organomegaly was detected in 6 (18%) of 33 patients, and bone mass loss was detected in 11 (37%) of 30 patients, 3 cases of which corresponded to osteoporosis. It is of note that bone mass

loss was detected at ages of less than 45 years in 9 (82%) of 11 patients (Table I). No patients presenting with bone sclerosis were identified (Table I).

Skin histology

Abnormally increased numbers of mature-appearing dermal MCs with a predominantly round/polygonal shape and fully granulated cytoplasm, either forming large aggregates of more than 15 MCs per cluster (94%) or distributed in a scattered fashion (6%), which is consistent with cutaneous involvement by mastocytosis, were found in every patient. In most (72%) patients dermal binucleated or polynucleated promastocytes were also identified, and variable numbers of eosinophils, lymphocytes, or polymorphonuclear cells were observed within the MC infiltrate in a subset of patients (9/33 [27%]). Overall, 3 different histopathologic patterns were recognized (Fig 2): (1) an interstitial/perivascular pattern ($n = 14$) characterized by MC infiltrates forming strands between collagen bundles and surrounding blood vessels, predominantly in the papillary dermis and upper reticular dermis; (2) a nodular pattern ($n = 8$) consisting of dense aggregates of MCs forming large and well-delimited nests of MCs mainly located within the reticular dermis and usually accompanied by variable numbers of scattered MCs after an interstitial distribution and also forming strands between collagen bundles; and (3) a sheet-like pattern ($n = 11$) with a diffuse MC infiltrate involving most of the papillary and reticular dermis, which eventually extended deep into the subcutaneous tissue. Immunohistochemical staining for CD25 showed weak to moderate membrane positivity in only 4 (17%) of 24 cases tested; in turn, CD30 was positive in 16 (70%) of 23 cases tested, with a weak ($n = 10$) to moderate/strong ($n = 6$) pattern of expression.

Morphologic, immunophenotypic, and molecular characteristics of BM MCs

BM MCs from all 33 patients systematically showed a round and well-granulated mature appearance, with a greater size than normal MCs from subjects without mastocytosis (Fig 3). Examination of BM sections revealed 3 main patterns of MC infiltration: (1) an interstitial pattern ($n = 4$) characterized by loosely scattered BM MCs without forming aggregates, (2) a focal pattern ($n = 24$) characterized by compact BM MC aggregates accompanied by a variable number of loosely scattered MCs, and (3) a diffuse pattern ($n = 4$) consisting of a diffuse-compact infiltration of the BM. Of note, BM MCs from cases with an interstitial or focal histologic pattern of BM infiltration typically showed an intertrabecular localization, with only 4 patients also showing perivascular distribution of BM MCs. Overall, dense compact MC aggregates (eg, >15 MCs per cluster) were detected in 21 (66%) of 32 patients and subdiagnostic aggregates (eg, median of 6 MCs per cluster; range, 2–14) in another 7 (22%) patients (Fig 3 and Table II). Cytologic examination of BM smears revealed large MC aggregates within BM particles in 27 (82%) of 33 patients, including 8 without MC aggregates detectable by means of immunohistochemistry and 1 pediatric patient in whom a BM biopsy was not performed (Fig 3 and Table II). In 28 (85%) of 33 patients, clusters of 2 to 3 MCs were found outside BM particles, and in 4 (12%) patients MCs represented more than 20% of all nucleated cells in BM

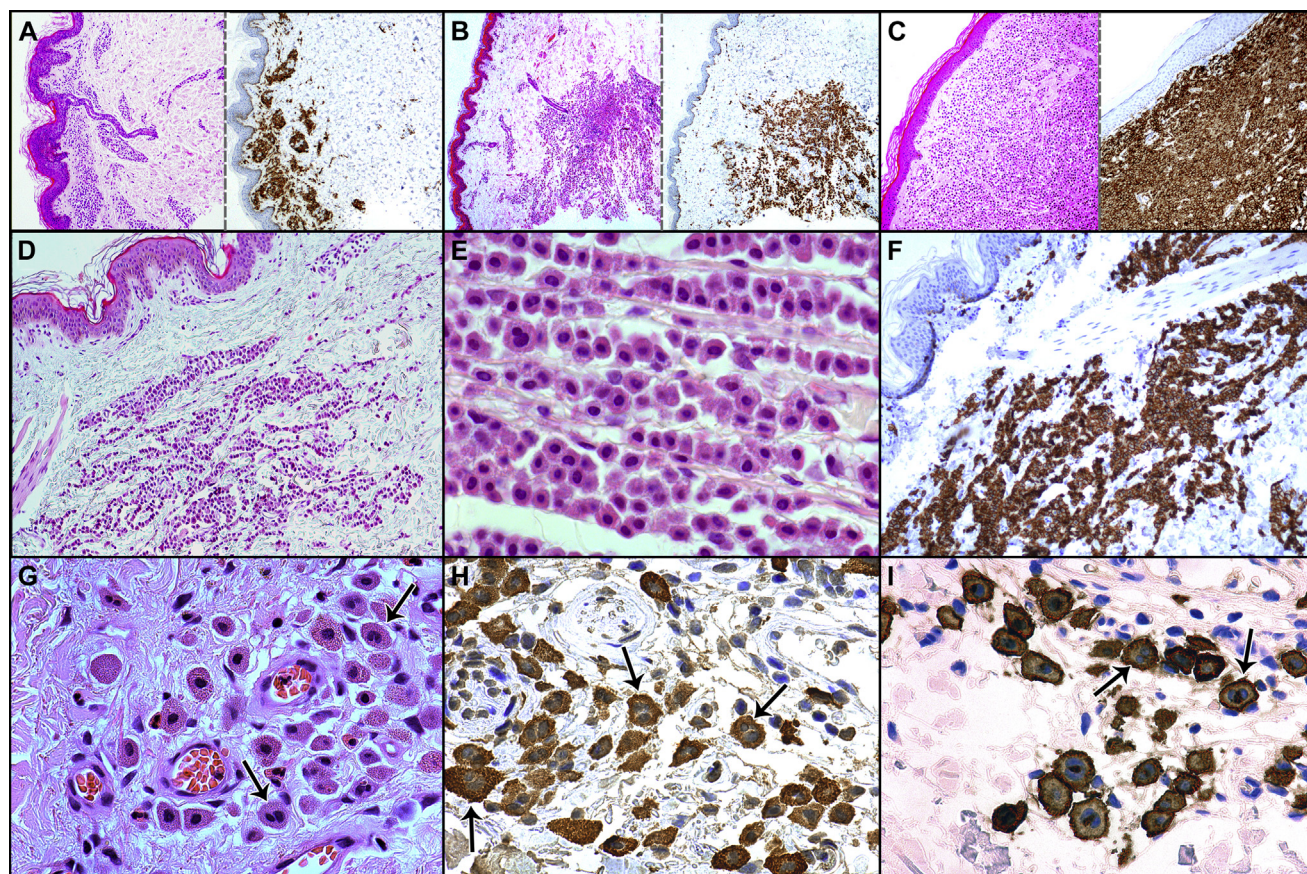


FIG 2. Representative histologic findings in skin lesion biopsy specimens from patients with WDSM. **A-C,** Skin biopsy specimens showing the 3 different histologic patterns of MC infiltration typically found in patients with WDSM (*left panels*, hematoxylin and eosin stain and $\times 40$ magnification; *right panels*, c-kit stain and $\times 40$ magnification): an interstitial/perivascular pattern characterized by dermal MC infiltrates within the interstitial spaces of the papillary dermis and surrounding the subepidermal vessels (Fig 1, A); a nodular pattern in which MCs are clustered with each other, forming large well-delimited dermal infiltrates accompanied by variable numbers of interstitial MCs (Fig 2, B); and a sheet-like pattern showing a diffuse MC infiltrate occupying the entire papillary and reticular dermis (Fig 2, C). **D-F,** Skin biopsy specimens showing round/polygonal skin MCs with the typical strand-like disposition (Fig 2, D, hematoxylin and eosin stain and $\times 100$ magnification; Fig 2, E, hematoxylin and eosin stain and $\times 600$ magnification; Fig 2, F, c-kit stain and $\times 100$ magnification). **G-I,** Skin biopsy specimens showing the round mature-like morphology of MCs and several bilobed promastocytes (*arrows*). The tryptase stain highlights the fully granulated cytoplasm of MCs. The c-kit stain shows a diffuse membranous and cytoplasmic dot-like pattern in MCs (Fig 2, G, hematoxylin and eosin stain and $\times 600$ magnification; Fig 2, H, tryptase stain and $\times 600$ magnification; Fig 2, I, c-kit stain and $\times 600$ magnification).

smears (Fig 3). In addition, hypergranulated promastocytes were identified in 3 (9%) patients, 2 of whom had a very high BM MC burden (Fig 3).

Immunophenotypically, BM MCs systematically lacked bright expression of both CD25 and CD2 (Fig 4); however, at least 1 of these markers was weakly expressed by 5% or more of the BM MCs in 7 (21%) patients (Table II). Compared with normal/reactive BM MCs, most patients exhibited underexpression of CD117 and FcεRI ($P < .001$), together with an abnormally increased light scatter (forward light scatter [FSC]/size and side light scatter [SSC]/granularity, $P < .001$) and overexpression of cytoplasmic carboxypeptidase A (CPA; $P = .03$, Table III and Fig 4). Aberrant expression of CD30, as assessed by means of flow cytometry (Fig 4) and immunohistochemistry, was found in 10 (71%) of 14 and 7 (50%) of 14 patients with WDSM tested, respectively.

KIT mutations were detected in only 10 (30%) of 33 patients, including the D816V ($n = 5$), I817V ($n = 1$), N819Y ($n = 1$), and K509I ($n = 3$) *KIT* mutations (Table II and see Table E1). Of note, those 3 patients with the K509I *KIT* mutation were members of the same family (2 sisters and their father), and they all had an associated gastrointestinal stromal tumor (GIST). In these 3 patients the K509I *KIT* mutation was detected in FACS-purified BM MCs, peripheral blood leukocytes, and, in the father, GIST cells, supporting a germline origin of the mutation. In turn, in the other *KIT*-mutated cases, the D816V, I817V, and N819Y *KIT* mutations were restricted to BM MCs.

sT levels

The median sT level at diagnosis was $9.3 \mu\text{g/L}$ (range, 3.1 – $271 \mu\text{g/L}$). Eight (24%) patients had sT levels of greater than $20 \mu\text{g/L}$,

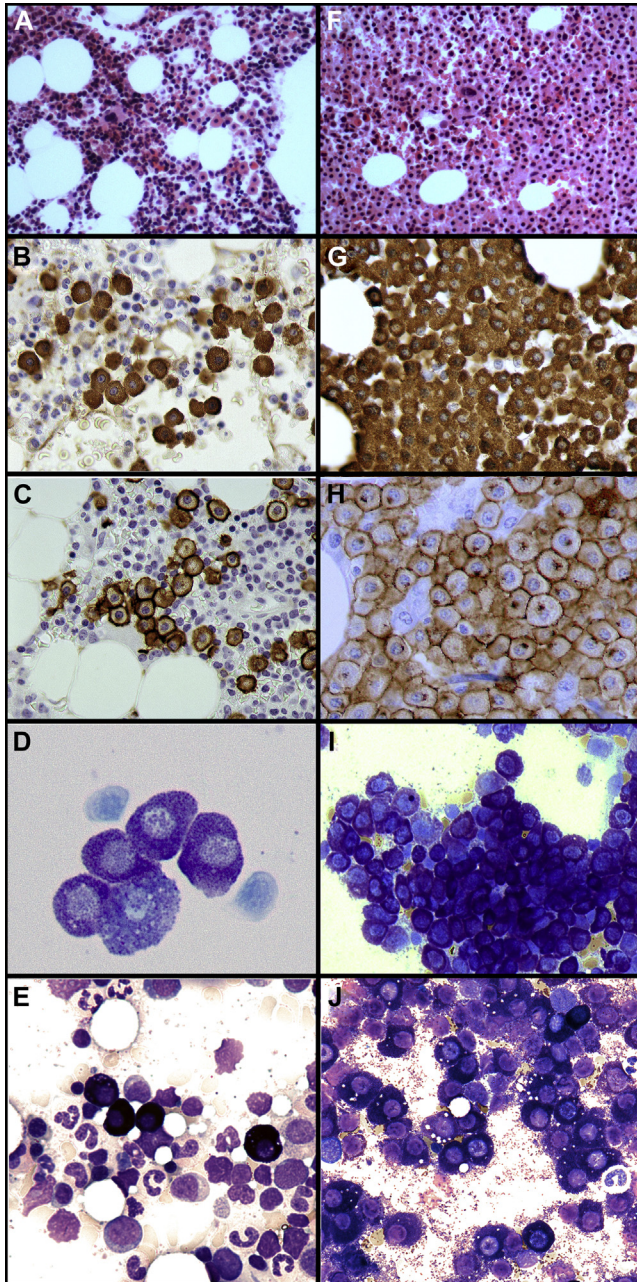


FIG 3. Images of representative histologic and cytomorphic BM features in patients with WDSM. **A-E**, BM sections (Fig 3, A-C) and smears (Fig 3, D-E) showing small clusters of round mature-like MCs in a patient with mild BM MC infiltration (Fig 3, A, hematoxylin and eosin stain and $\times 200$ magnification; Fig 3, B, tryptase stain and $\times 600$ magnification; Fig 3, C, c-kit stain and $\times 600$ magnification; Fig 3, D, toluidine blue stain and $\times 1000$ magnification; Fig 3, E, May-Grünwald-Giemsa stain and $\times 600$ magnification). **F-J**, BM sections (Fig 3, F-H) and smears (Fig 3, I-J) showing a marked increase of round and well-granulated MCs diffusely infiltrating the BM in a patient fulfilling the criteria for MCL (Fig 3, F, hematoxylin and eosin stain and $\times 200$ magnification; Fig 3, G, tryptase stain and $\times 600$ magnification; Fig 3, H, c-kit stain and $\times 600$ magnification; Fig 3, I, toluidine blue stain and $\times 600$ magnification; Fig 3, J, May-Grünwald-Giemsa stain and $\times 600$ magnification).

whereas 19 (58%) patients had normal sT levels ($<11.4 \mu\text{g/L}$, Table II). Overall, sT levels showed a significant correlation with the percentage of BM MCs detected by means of flow cytometry

($R = 0.816$, $P < .001$), with the highest sT values corresponding to patients with high BM MC burden (Table II).

WHO 2008 criteria for SM

Despite most of the 33 patients having multiorgan (eg, systemic) involvement (eg, skin lesions plus BM aggregates), only 9 (27%) patients could be given a diagnosis of SM strictly based on the WHO 2008 criteria. These 9 patients had BM MC aggregates together with 1 ($n = 7$) or 2 ($n = 2$) minor criteria; according to the WHO 2008 classification of SM, 4 of these 9 patients corresponded to ISM, 1 to SSM, and 4 to MCL (Table II). The other 24 (73%) patients did not meet enough criteria for SM and were eventually classified as having CM according to the same WHO 2008 classification of mastocytosis, despite the presence of BM aggregates of 15 or more MCs in the absence of other minor criteria in 12 of this subgroup of 24 patients. Another 7 patients fulfilled 1 to 2 minor criteria in the absence of MC aggregates in the BM biopsy specimen, and 4 patients did not fulfil any of the major or minor criteria for SM (Table II). The pediatric patient who fulfilled only 1 minor criterion (sT level $>20 \mu\text{g/L}$) for SM was unclassifiable according to WHO recommendations because he underwent BM aspiration without biopsy, and thus the presence of compact MC aggregates in BM sections could not be evaluated, even though he showed BM MC aggregates in BM smears (Table II). Patients classified as having CM after the WHO 2008 classification had MPCM and normal sT levels ($<11.5 \mu\text{g/L}$) more frequently than those fulfilling the WHO 2008 criteria for SM (96% vs 56% [$P = .01$] and 75% vs 22% [$P = .009$], respectively); by contrast, no significant differences were observed regarding MC mediator release symptoms between both groups of patients.

Pattern of inactivation of chromosome X by using HUMARA in BM MCs

MC clonality was established by using HUMARA on highly purified BM MCs from 8 female patients who systematically lacked *KIT* mutations. These included 6 patients classified as having CM when the WHO 2008 diagnostic criteria were strictly applied, 1 patient with ISM, and 1 patient who fulfilled the criteria for MCL (Table II). By contrast, a polyclonal HUMARA pattern was detected in 6 patients, which included 3 patients with CM, 1 patient with SSM, and 2 patients with MCL; one of these latter 2 patients corresponded to a female subjects with the K509I (germline) *KIT* mutation (Table II).

DISCUSSION

Currently, it is well established that mutations involving codon 816 of *KIT*, abnormal morphology, and strong expression of CD25 ($\text{CD25}^{+ \text{bright}}$) can be detected in BM MCs from the great majority of patients with SM provided that highly sensitive diagnostic methods are used.^{6,9,21,22} Despite this, patients with MCs who lack such biological features have been sporadically reported in the literature. Thus in 2004, Akin et al⁴ reported a case of ASM associated with CD25^{-} BM MCs with a normal morphologic appearance and a germline mutation (F522C) involving the transmembrane domain of *KIT*. This unusual variant of mastocytosis was termed WDSM based on the mature morphology of MCs and their well-differentiated phenotype.

TABLE II. WHO criteria for SM and MC clonality assessment in patients with WDSM

CD25/CD2 expression (% of positive cells)											
Patient ID	Sex	MC aggregates in BM sections/ smears	Well-differentiated MC morphology	CD25/CD2 expression (% of positive cells)	BM MCs by FC (%)	<i>KIT</i> mutation	sT (μg/L)	sT >20 μg/L	WHO criteria for SM	WHO diagnosis	HUMARA
1	F	Yes/Yes	Yes	—/—	0.02	No	11	No	1 major	CM	Polyclonal
2	M	Yes/No	Yes	—/—	0.009	No	7.9	No	1 major	CM	NA
3	F	Yes/Yes	Yes	—/—	0.1	No	6.5	No	1 major	CM	Not tested
4	F	Yes/Yes	Yes	—/—	0.077	No	7.3	No	1 major	CM	Clonal
5	F	Yes/No	Yes	—/—	1.05	D816V	11.5	No	1 major + 1 minor	SM (ISM)	Not tested
6	F	Yes/Yes	Yes	—/—	0.3	No	12.5	No	1 major	CM	Clonal
7	F	No*/Yes	Yes	—/—	0.0092	No	6.6	No	No	CM	Clonal
8	F	Yes/Yes	Yes	—/—	0.12	No	12.3	No	1 major	CM	Clonal
9	F	No*/No	Yes	—/—	0.008	D816V	3.2	No	1 minor	CM	Not tested
10	F	No/No	Yes	—/+low (10%)	0.001	D816V	3.1	No	2 minor	CM	Not tested
11	F	Yes/Yes	Yes	—/+low (100%)	14	No	244	Yes	1 major + 2 minor	SM (MCL)	Polyclonal
12	F	Yes/Yes	Yes	—/—	0.02	No	17.8	No	1 major	CM	Clonal
13	M	Yes/No	Yes	—/—	0.002	D816V	6.5	No	1 major + 1 minor	SM (ISM)	NA
14	F	Yes/Yes	Yes	—/—	0.013	N819Y	15	No	1 major	CM	Not tested
15	F	Yes/Yes	Yes	—/—	0.02	No	5.2	No	1 major	CM	Homozygous
16	F	Yes/Yes	Yes	—/+low (96%)	0.15	No	193	Yes	1 major + 2 minor	SM (SSM)	Polyclonal
17	F	No*/No	Yes	—/—	0.008	I817V	3.6	No	No	CM	Not tested
18	F	Yes/Yes	Yes	—/+low (27%)	0.028	No	4.5	No	1 major + 1 minor	SM (ISM)	Clonal
19	F	No*/Yes	Yes	—/—	0.034	D816V	8.2	No	1 minor	CM	Not tested
20	F	Yes/Yes	Yes	—/—	11.7	No	271	Yes	1 major + 1 minor	SM (MCL)	Clonal
21	F	Yes/Yes	Yes	—/—	0.015	No	6.5	No	1 major	CM	Clonal
22	F	Yes/Yes	Yes	—/—	0.04	No	18.2	No	1 major	CM	Not tested
23	M	Yes/Yes	Yes	—/—	7	K509I	84.9	Yes	1 major + 1 minor	SM (MCL)	NA
24	F	Yes/Yes	Yes	—/—	0.78	K509I	40.4	Yes	1 major + 1 minor	SM (ISM)	Not tested
25	F	Yes/Yes	Yes	—/—	11	K509I	108	Yes	1 major + 1 minor	SM (MCL)	Polyclonal
26	M	Yes/Yes	Yes	—/—	0.024	No	5.2	No	1 major	CM	NA
27	F	No*/Yes	Yes	—/—	0.018	No	5.7	No	No	CM	Polyclonal
28	F	No/Yes	Yes	+low (40%)/ +low (40%)	0.01	No	6.2	No	1 minor	CM	Polyclonal
29	F	No/Yes	Yes	+low (100%)/—	0.02	No	9.3	No	1 minor	CM	Not tested
30	F	No*/Yes	Yes	+low (100%)/—	0.016	No	6.0	No	1 minor	CM	Not tested
31	F	No*/Yes	Yes	—/—	0.04	No	11	No	No	CM	Not tested
32	M	No/Yes	Yes	—/—	0.3	No	37.5	Yes	1 minor	CM	NA
33	M	NA/Yes	Yes	—/—	0.2	No	121	Yes	1 minor	NA	NA
Total (%)	F: 27 (82%)	21/27 (66%/82%)	33 (100%)	3/5 (9%/15%)	0.028 (0.001-14)‡	10 (30%)†	9.3 (3.1-271)‡	8 (24%)	9/33§ (27%)	9/33§ (27%)	8/14 (57%)

F, Female; FC, flow cytometry; M, male; NA, not available.

*Subdiagnostic aggregates of 2 to 14 MCs per cluster in BM sections.

†D816V in 5 (50%) of 10 mutated cases.

‡Expressed in medians (ranges).

§Number of patients fulfilling the WHO 2008 criteria for SM.

||Number of patients showing a clonal pattern of inactivation of chromosome X by using HUMARA from all female patients tested.

Since then, only a few studies have reported small numbers of patients with SM with similar morphologic and immunophenotypic patterns. Among such studies, the Spanish Network on Mastocytosis reported 9 patients classified as having WDSM who had a low frequency of exon 17 *KIT* mutations.⁶ In a later study including 2 additional patients (n = 11), we further showed that BM MCs from patients with WDSM frequently lacked CD25, CD2, or both, whereas they typically exhibited abnormally increased expression of cytoplasmic proteases versus other typical ISM cases⁸; more recently, BM MCs from 8 of 9 patients with WDSM were shown to aberrantly express CD30, a trait held in common with other cases of ISM and ASM.²³ In parallel, dramatic responses to imatinib therapy have been reported in the patient with the F522C *KIT* mutation,⁴ in another patient with WDSM who had no demonstrable *KIT* mutations,¹⁰ and,

more recently, in 3 patients with the K509I (germline) *KIT* mutation.^{11,12} Altogether, these observations support the notion that WDSM represents a subtype of SM that displays unique morphologic, phenotypic, and molecular features with potential therapeutic implications. Despite this, a precise description of the prevalence and clinical characteristics of WDSM, as well as a clear definition of the diagnostic criteria for this subtype of SM, are still lacking.

Here we describe the clinical, histopathologic, and molecular characteristics of a group of 33 patients with histologically proved MIS selected on the basis of an apparently normal morphology of MCs together with a CD25^{−/low} and CD2^{−/low} immunophenotype. Because all 33 patients had additional features that provided evidence for systemic involvement by mastocytosis, we conclude that they could be categorized as having WDSM, despite only a

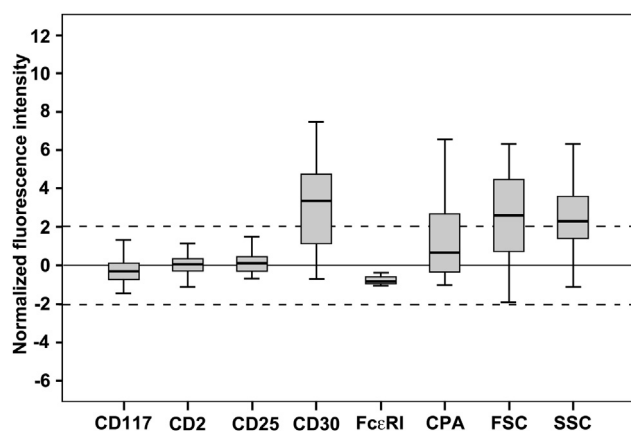


FIG 4. Flow cytometric immunophenotypic profile of BM MCs from patients with WDSM gated as CD117⁺high/CD45⁺ cells with intermediate to high scatter. *Solid and dotted horizontal lines* represent the mean $\pm$ 2 SDs (95% CI), respectively, of normalized fluorescence intensity values per BM MC for each immunophenotypic marker found in normal/reactive BM MCs from a group of patients without mastocytosis (n = 36) undergoing BM aspiration for reasons other than mastocytosis.

minor fraction meeting the current WHO 2008 criteria for SM. Of note, combined use of the above 2 criteria allowed us to exclude patients with ISM with round hypergranulated MCs and strong CD25 expression,^{9,24} patients with myelomastocytic leukemia and acute MCL who had CD25[−] MCs with an immature morphology,^{2,25} and patients in whom normal MCs coexist with a small population of morphologically abnormal CD25⁺bright MCs, as typically detected among patients with ISM with low tumor burden.^{8,9,26–28}

Despite CD25 and CD2 being systematically absent or expressed at low levels on the surface membrane, BM MCs from our patients could still be clearly distinguished from normal BM MCs based on their increased light scatter and abnormally high expression of CD30 and cytoplasmic CPA, as assessed by using flow cytometry, as previously suggested in small patient series.^{8,23} Of note, the light scatter properties and amount of cytoplasmic CPA cannot be evaluated by using immunohistochemistry; in addition, this latter technique also does not detect some CD30⁺ cases,²³ which supports an advantage for multiparameter flow cytometry versus conventional immunohistochemistry for an extended phenotypic analysis of BM MCs from patients with suspected WDSM. The greater amount of cytoplasmic proteases could help explain both the hypergranulated morphology of BM MCs in patients with WDSM and their abnormally increased forward FSC and SSC values versus normal BM MCs, as well as the lower than expected sT levels based on the extent of BM and skin involvement by MCs observed in our series (eg, 2 of 4 patients with MCL and 3 of 4 patients with ISM had sT levels <200 μ g/L and <20 μ g/L, respectively). Such findings probably reflect a reduced chronic release of tryptase per MC in patients with WDSM, which could be associated with the low frequency of the D816V *KIT* mutation, as discussed below. Another factor that might contribute to the unexpectedly low sT levels found among our patients with WDSM is the lower frequency of perivascular BM MC aggregates (vs typical SM),²⁹ which could make entrance of the tryptase released from these MCs into the systemic circulation more difficult.

TABLE III. Proposed diagnostic criteria for WDSM*

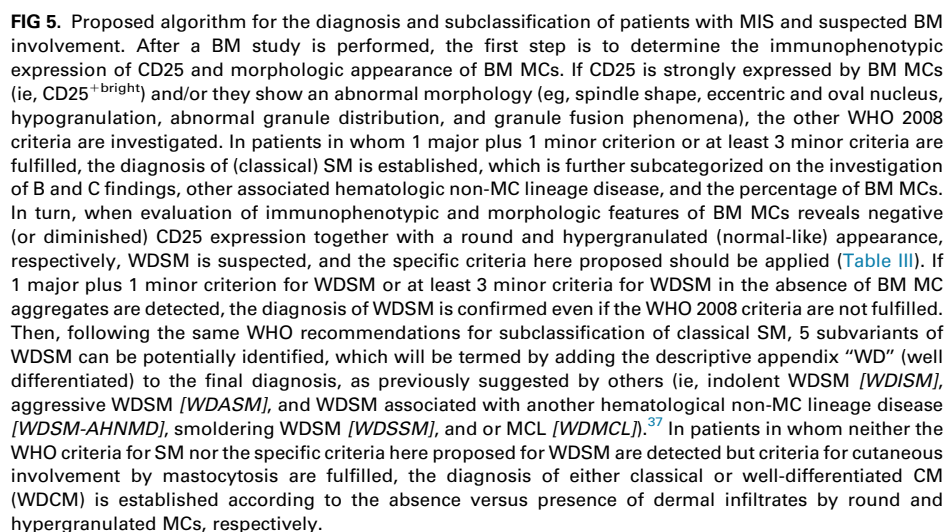
Major criterion
Compact MC aggregates in BM sections or smears
Minor criteria
Clustering of BM MCs outside BM particles forming groups of ≥ 2 MCs
Aberrant expression of CD30, overexpression of cytoplasmic proteases (CPA, tryptase, or both), or both by means of flow cytometry
Detection of mutations involving any codon of <i>KIT</i> or a clonal HUMARA pattern
Association of female sex with either pediatric disease onset or familial aggregation in adults

*At least the major plus 1 minor criterion or 3 minor criteria in the absence of the major criterion should be fulfilled.

Despite their apparently “normal” morphology, BM MCs from our patients also showed unique cytologic features, including a greater size than normal MCs, which is in line with their increased FSC on flow cytometric grounds, and a tendency to cluster with each other, forming small groups of a few MCs to focal MC aggregates, such as tryptase-positive round cell infiltrates in the BM,²⁴ which more rarely lead to a diffuse pattern of BM infiltration. Immunohistochemical staining of MCs for CD117 in the absence of both CD25 and CD34 (data not shown) contributed to the histologic differential diagnosis with other entities that might present with tryptase-positive round cell infiltrates in the BM, such as myelomastocytic leukemia, MCL, ISM, basophilic leukemia secondary to chronic myeloid leukemia, or CD117⁺ acute myeloid leukemia.^{24,30}

Among all biological features of pathologic MCs from our patients with WDSM, the unexpectedly low frequency of D816V *KIT* mutation and other exon 17 *KIT* mutations is probably the most relevant because it can render a significant proportion of patients sensitive to TKI therapy. In fact, complete responses to imatinib have been almost exclusively seen among D816V-negative patients with SM who carry other molecular alterations involving the transmembrane (eg, F522C) and juxta-membrane (eg, V560G and K509I) domains of *KIT*^{4,11,12,31–33} or no *KIT* mutation associated or not with mutations involving genes other than *KIT* (eg, FIP1L1/PDGFR α fusion gene).^{10,32,34} Fully in line with this, one third of our *KIT*-mutated patients with WDSM had the K509I mutation located at exon 9 in the extracellular juxtamembrane domain of *KIT*, which has been already reported in 4 patients with SM who were classified as having ASM, SSM evolving to ASM, MCL, and ISM, respectively,^{11,12,33} according to the WHO 2008 diagnostic criteria, and who had dramatic responses to imatinib. Interestingly, BM MCs from all 4 of these patients displayed a well-differentiated morphology, and CD25 levels were negative in 2 of 2 patients analyzed. Moreover, a complete response to imatinib has already been reported in one of the patients with WDSM included in our study who had clonal BM MCs with no *KIT* mutations (case 2)¹⁰ and in another 3 of 8 cases here analyzed included in the National Institutes of Health code NCT01297777 treatment protocol (data not shown).

Despite most patients (70%) from our study having wild-type *KIT*, MC clonality was established in a significant proportion of them through HUMARA, supporting the existence of as-yet-undiscovered mutations in genes other than *KIT* that could contribute to the clonal nature of the disease in a significant fraction of patients with WDSM. At the same time, we also



Despite the unique and common features described above, our patients with WDSM presented with a highly variable tumor burden. Thus although most patients had relatively low levels of

BM MC infiltration at the time of the BM study, approximately 15% of them had a markedly high BM MC burden, fulfilling the WHO 2008 criteria for either SSM or MCL. It is noteworthy that most of the latter patients with MCL had a slow and long-lasting disease course in the absence of WHO C-findings, fulfilling recently proposed criteria for chronic MCL.³⁶ The evidence for a prior long-term history of MIS in these patients, as well as in other patients with advanced WDSM reported in the literature,^{4,11,12,33} suggests that all of them had an indolent form of the disease at onset, which progressed over time. This provides a rationale for an early diagnostic work-up in patients with suspected WDSM and a close follow-up if WDSM is confirmed, particularly in patients in whom multilineal or germline *KIT* mutations are detected.

Although final evidence for WDSM is attained through morphologic, immunophenotypic, and molecular assessment of BM MCs, WDSM was already suspected in adult female patients with a pediatric onset of skin lesions in the context of a familial history of mastocytosis and maculopapular skin lesions, usually showing a central body distribution with frequent involvement of the neck by lesions with a (micro)nodular macroscopic appearance. Despite this, in our patients skin disease was heterogeneous, with patients also presenting with solitary or multiple (macro) nodular lesions or even DCM among those with the K509I *KIT* mutation, as also described by others.^{11,12,33} Despite the heterogeneity of cutaneous involvement, the unique mature appearance of MCs could also be systematically demonstrated in the skin of patients with WDSM; there MCs were frequently found in association with variable numbers of promastocytes and to form strands between collagen bundles, features that are rarely observed in other patients with mastocytosis. Further studies in which such features are investigated in skin lesions of children with MIS are needed to determine their potential predictive value for the persistence of mastocytosis into adulthood and its further potential correlation with underlying WDSM, particularly among female patients and those with a familial background of mastocytosis.

The hypothesis that WDSM can already emerge in childhood as a systemic disease in nature that persists into adulthood is supported by the fact that 2 of our patients had already received diagnoses of WDSM at young ages (2 and 12 years, respectively).

In turn, our study also confirms previous observations about spontaneous regression of skin involvement in some patients with WDSM before demonstration of the clonal nature of the disease in the BM,⁴ which would support a long-term follow-up of those children with a prior history of MIS and skin biopsy features suggesting WDSM, even after skin lesions eventually disappear. Finally, appropriate identification of patients with WDSM might also be of clinical relevance for genetic counseling purposes, given the high rate of familial cases described here.

Because current WHO criteria for SM only allow establishment of the systemic nature of the disease in a fraction of all patients with WDSM, additional diagnostic criteria for SM are needed for those patients presenting with MIS in association with BM infiltration by mature-appearing MCs (usually forming compact BM MC aggregates of >15 MCs per cluster) in the absence of bright CD25 expression by means of flow cytometry. Based on our results, specific minor diagnostic criteria for WDSM could be selected from those features found to be typically associated with the patients with WDSM described here. These include (1) adult women with a pediatric onset or familial aggregation in

first-degree relatives, (2) clustering of BM MCs outside BM particles forming pairs or triplets of MCs, (3) aberrant expression by BM MCs of CD30 together with overexpression of cytoplasmic proteases (CPA and tryptase) by using flow cytometry, and (4) mutations involving any codon of the *KIT* gene (or a clonal pattern of inactivation of the chromosome X by using HUMARA). Once combined with the presence of MC aggregates in either BM sections or BM smears, these criteria would have allowed correct diagnosis of all patients with WDSM studied here if the same scoring recommendations as those proposed by the WHO for classical SM (eg, presence of BM MC aggregates and 1 minor criterion or coexistence of at least 3 of 4 minor criteria here proposed in the absence of BM MC aggregates) had been followed (Table III). Following these newly proposed diagnostic criteria for WDSM and the WHO recommendations for the subclassification of SM, patients with WDSM could then be potentially subclassified into indolent WDSM, aggressive WDSM (WDASM), and WDSM associated with another hematological non-MC lineage disease (WDSM-AHNMD), smoldering WDSM (WDSM), or MCL (WDMCL; Fig 5) categories.³⁷

In summary, we show that despite WDSM constituting a biologically distinct rare subgroup of SM that can be misdiagnosed often as CM strictly based on the current WHO 2008 diagnostic criteria for SM, it is still highly heterogeneous both on clinical and molecular grounds. On clinical grounds, WDSM appears to show the entire clinical spectrum of typical CD25⁺ and D816V-positive SM, as supported by the existence of indolent, smoldering, aggressive, and leukemic WDSM cases. In turn, from a practical point of view, identification of the molecular subgroups of WDSM as defined by the presence versus absence of exon 17 *KIT* mutations appears to be of utmost relevance because of the higher probability of response to TKI therapy of patients carrying *KIT* mutations at exons other than exon 17 reported in the literature. Because WDSM only accounts for a very small fraction of all cases of SM (approximately 5%), multicenter studies including larger series of patients are needed to confirm our findings, to define and validate the new diagnostic criteria here proposed and the current WHO criteria for subclassification of this rare subgroup of patients with SM, and to establish prospective clinical trials to evaluate the effects of TKI therapy in the above-defined molecular subgroups of patients with WDSM.

Clinical implications: WDSM represents a rare subtype of SM with unique clinical, biological, and molecular features that requires specific diagnostic criteria for systemic involvement and will potentially benefit from *KIT* tyrosine kinase-targeted therapy.

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TABLE E1. *KIT* mutation analysis performed in patients with WDSM

Patient ID	<i>KIT</i> mutation	<i>KIT</i> exons investigated (sequenced)
1	No	2, 8, 9, 10, 11, 17
2	No	2, 9, 10, 11, 13, 14, 15, 17
3	No	10, 11, 17
4	No	2, 8, 9, 10, 11, 17
5	D816V	17
6	No	2, 9, 10, 11, 13, 14, 15, 17
7	No	2, 9, 10, 11, 13, 14, 15, 17
8	No	2, 9, 10, 11, 17
9	D816V	17
10	D816V	17
11	No	2, 8, 9, 10, 11, 17
12	No	2, 9, 10, 11, 17
13	D816V	17
14	N819Y	17
15	No	8, 9, 10, 11, 17
16	No	2, 9, 10, 11, 17
17	I817V	17
18	No	8, 9, 10, 11, 17
19	D816V	17
20	No	2, 9, 10, 11, 13, 14, 15, 17
21	No	2, 9, 10, 11, 13, 14, 15, 17
22	No	2, 9, 10, 11, 17
23	K509I	9, 17
24	K509I	9, 17
25	K509I	9, 17
26	No	8, 9, 10, 11, 17
27	No	2, 8, 9, 10, 11, 17
28	No	2, 9, 11, 17
29	No	8, 11, 17
30	No	2, 8, 9, 10, 11, 17
31	No	2, 8, 9, 10, 11, 17
32	No	2, 9, 10, 11, 14, 15, 17
33	No	2, 9, 11, 17