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 2016;137:168-78.)

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 mastocytosis[1]: 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

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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

than 20 μg/L in the absence of other minor WHO 2008 diagnostic 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 criteria[2]; (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%])