

Bone Marrow Expression of Mast Cell Disorders



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KEYWORDS

- Mast cells • Mastocytosis • Flow cytometry • Bone marrow • Cell purification
- Diagnosis • KIT mutation • Prognosis

KEY POINTS

- Mast cell disorders constitute a group of heterogeneous diseases that should be managed in specialized reference centers, in order to increase patient access to high-quality diagnostic tests and treatment.
- To establish the correct diagnosis and prognosis of the disease, highly specialized laboratory diagnostic tests should be applied in the diagnostic workup in suspected systemic mastocytosis.
- Multiparameter flow cytometry stands as the most sensitive technique to determine the grade of involvement of the bone marrow, together with the exact diagnosis, subtype, and prognosis of mastocytosis.
- Sensitive approaches for detecting KIT mutation in highly purified bone marrow mast cells are mandatory to avoid false negative results, moreover when a pathologic low bone marrow mast cell burden is suspected.

INTRODUCTION

The World Health Organization (WHO) recently updated the classification of mastocytosis^{1–3} in the following categories:

- I. Cutaneous mastocytosis (CM) when it is limited to the skin;
- II. Systemic mastocytosis (SM) when abnormal mast cells (MCs) are located in extracutaneous organs, including the bone marrow (BM); it is divided into indolent

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SM (ISM), smoldering SM, SM with an associated hematologic (non-MC lineage) neoplasm (SM-AHN), aggressive SM (ASM), and mast cell leukemia (MCL);

III. MC sarcoma. Furthermore, 2 other subvariants of mastocytosis have been recognized:

1. Well-differentiated forms with cutaneous and systemic (WDSM) involvement⁴; these forms show unique features that require specific diagnostic criteria;
2. ISM in the absence of skin lesions (ISMs–).^{5–7}

According to the WHO criteria,^{2,3} the diagnosis of SM is based on the coexistence of one major criterion (presence of multifocal dense aggregates of ≥ 15 MCs in BM biopsies and/or in sections of other extracutaneous organs) plus one minor criterion or simultaneous detection of 4 minor criteria:

- A. Identification of greater than 25% of atypical MCs on BM smears or infiltrates of spindle-shaped MCs on sections of visceral organs,
- B. KIT point mutation at codon 816 in the BM or another extracutaneous organ,
- C. MCs in BM or blood or another extracutaneous organ exhibit CD2 and/or CD25,
- D. Baseline serum tryptase levels greater than 20 ng/mL.

In clinical practice, the suspicion of an underlying MC disorder is based on the presence of the typical skin lesions of mastocytosis or in patients without skin lesions presenting with systemic symptoms of MC activation (MCA) (eg, anaphylaxis).⁸

Despite the great relevance and efficiency of the WHO criteria in the diagnosis of SM, frequently in nonaggressive categories of SM, MCs represent a very small proportion of all nucleated BM cells ($<10^{-3}$ BM MCs, as assessed by flow cytometry).^{9,10} Along this line, BM MC aggregates are not found in 30% to 54% of the ISMs– patients and serum tryptase levels are frequently less than 20 ng/mL in 31% to 38% of the ISMs– cases (anaphylaxis elicited by other triggers vs insects, respectively).¹¹ Therefore, it is mandatory to apply highly sensitive and specific methodological approaches to the study of BM MCs, including detailed cytologic analysis of BM smears, flow cytometry immunophenotyping using specific strategies for detecting MCs present at low frequencies,^{12,13} detection of KIT mutations in highly purified BM MCs,¹⁴ as well as the rational combination of the SM criteria in the routine diagnosis of SM,^{10,15} in order to improve the diagnostic efficiency and quality as previously reported.¹⁶

Of note, the sensitivity and specificity of the methodological approaches used in the diagnosis of ISMs– contribute to explain the differences in the percentage of SM among adults presenting with systemic symptoms of MCA in the absence of skin lesions described in different reports.^{7,17–19} In addition, different terms such as (mono) clonal MC activation syndrome (MMAS)^{8,18,19} or primary MC activation syndrome²⁰ or clonal MC activation disorder (c-MCAD)⁷ emerged to name those cases who do not meet the WHO criteria for SM, despite KIT mutated clonal MCs that usually express CD25 are found (Fig. 1).

The mast cell activation syndrome (MCAS) includes a heterogeneous group of diseases that are characterized by systemic symptoms secondary to the release of MC mediators that may or may not have a known trigger, may or may not present specific immunoglobulin E (IgE) antibodies in response to that trigger, and are associated with normal or elevated baseline tryptase levels in the absence of skin lesions of mastocytosis.^{8,21} The diagnosis and classification of MCAS are based on previous described clinical and laboratory criteria.⁸ The Spanish Network of Mastocytosis (REMA) supports that the most relevant fact to classify the MCAS is the presence versus absence of clonal MCs (based on the expression of CD25⁺ and/or KIT mutation).²¹ When

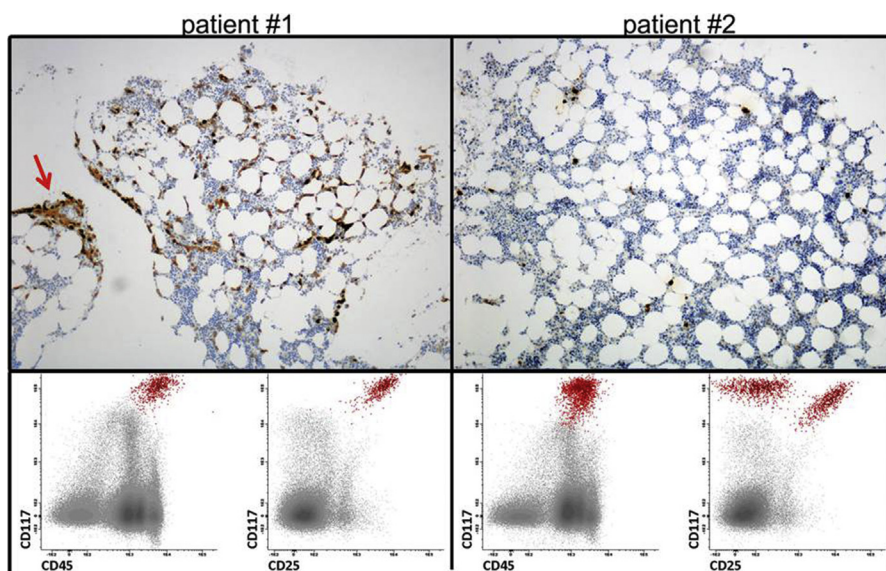


Fig. 1. BM sections showed increased numbers of interstitial MCs and MC aggregates (red arrow) in patient 1 (ISM—), but scattered MCs in the absence of aggregates in patient 2 (c-MCAS/MMAS); (upper panels) tryptase stain, $\times 400$. BM aspirate analysis by flow cytometry showed 0.09%/0.002% of BMMCs (red dots) in patients 1 and 2, respectively. Expression of CD25 was detected in all BMMCs in patient 1, whereas only 53% in patient 2.

MCAS diagnostic criteria are fulfilled but there is no evidence of clonality, nonclonal MCAS should be considered. Along this line, the European Competence Network on Mastocytosis recommends using the REMA score⁷ to predict the presence of clonal MCs in the BM of patients diagnosed of MCAS, in order to select those cases that should undergo a complete BM study.

The clonal nature of MCs in mastocytosis or clonal MC disorders can be established through demonstration of KIT mutations in lesional skin and/or BM cells,^{14,22–25} resulting the KIT D816V as the most frequently mutation found in SM.

BM is the most appropriate site for screening systemic involvement in mastocytosis because it is the most frequently documented site of extracutaneous involvement, resulting in the expansion and accumulation of aberrant MCs in distinct tissues.^{2,26}

Because diagnosis of mastocytosis may be a challenge because of the wide variety of clinical presentation, abnormal morphology of MC, and variation in histologic features, a thorough histologic, immunohistochemical, and morphologic BM examination remains an important feature in the diagnostic workup in suspected SM.²⁷

Bone Marrow Histopathologic Features of Mast Cell Disorders

The presence of multifocal, dense aggregates of ≥ 15 MCs in tissue sections from biopsy specimens from BM and/or other extracutaneous organs is highly specific of mastocytosis. In addition, mastocytosis may also present with a predominantly diffuse or a mixed (diffuse and focal) infiltration pattern.^{28,29} Depending on the number and distribution of MCs in the BM, different histologic patterns can be observed.²⁸ Accordingly, whereas ASM and MCL cases systematically show a dense, diffuse MC infiltration, ISM may have different patterns of BM involvement ranging from an increase of interstitial MCs without evidence of compact MC aggregates, to focal, dense MC aggregates with or without a diffuse component.³⁰

Bone Marrow Immunohistochemistry

Immunohistochemical staining of BM biopsy sections with tryptase is recommended for MC-detection and evaluation of BM MC infiltration,²⁸ resulting more informative than using only conventional stains (eg, hematoxylin and eosin or Giemsa).³¹

c-Kit staining is routinely used to identify both normal and abnormal MCs, whereas CD25 is a reliable and specific marker for SM MCs.³⁰ Despite this, its utility is limited in cases lacking MC aggregates (resulting difficult to differentiate between abnormal MCs and CD25-positive T lymphocytes), and in CD25-negative WDSM. In addition, expression of CD30 has been reported to be a potentially useful prognostic marker because it is strongly expressed by MCs from poor-prognosis categories of mastocytosis, ASM and MCL.^{32,33}

Morphologic Expression of Mast Cell Disorders

MCs can be easily identified in toluidine blue-stained BM smears as red-purple elements over a blue background (**Fig. 2**). On the other hand, May-Grünwald-Giemsa stain allows a better cytomorphologic characterization of MCs (mainly outside the BM particles), as well as the identification of other morphologic features suspicious of a coexisting hematological malignancy. Wright and Giemsa stains of BM MCs of patients with clonal MCD show morphologic features such as the following:

- I. Metachromatic granulated blast and immature atypical MCs with bilobed or multilobed nuclei: promastocyte or atypical MC type II,
- II. More mature cells, which show an atypical morphology with prominent surface projections, frequently spindle-shaped, with a hypogranulated cytoplasm and/or an oval eccentric nucleus, atypical MC type I, and
- III. Typical mature (tissue) MC features, consisting of round or oval-shaped cells with a round central nucleus and a well-granulated cytoplasm.³⁴

Along this line, ASM and MCL typically show immature cytologic features such as a wide-sized heterogeneity, multilobed nuclei (promastocytes), or a poorly differentiated morphology (metachromatic blasts)^{10,34}; round or polygonal BM MCs may coexist with fusiform shapes in some SM cases,¹⁰ and WDSM BM MCs typically exhibit mature, increased size, round shaped with round nucleus, and fully granulated cytoplasm with relatively frequent degranulation phenomena, vacuoles.³⁵

Origin, Identification, and Enumeration of Bone Marrow Mast Cells by Flow Cytometry

MCs derive from BM hematopoietic progenitors and circulate through the vascular system as immature progenitors, before they migrate into peripheral tissues, where they complete the final stages of their maturation under the influence of a tissue-specific local microenvironment.³⁶

As stated previously, MCs represent an infrequent cell population in normal BM-aspirated samples ($0.008\% \pm 0.008\%$ of all nucleated cells in adult human BM).^{13,37} Although reactive BM samples and BM samples from patients with non-MC-related hematologic disorders may show slightly increased mean BM MC numbers ($0.027\% \pm 0.17\%$), in most instances, these cells represent less than 0.1% the whole BM cellularity.³⁸ Interestingly, despite the increased frequency of BM MCs found in reactive samples versus normal BM, few immunophenotypic differences have been described between normal and reactive MCs.^{13,37,39}

MCs strongly express CD117, CD203c, and the high-affinity receptor for IgE, FcεRI^{38,40} (**Fig. 3**); nevertheless, none of them is a specific marker for MC.^{41–43} In early

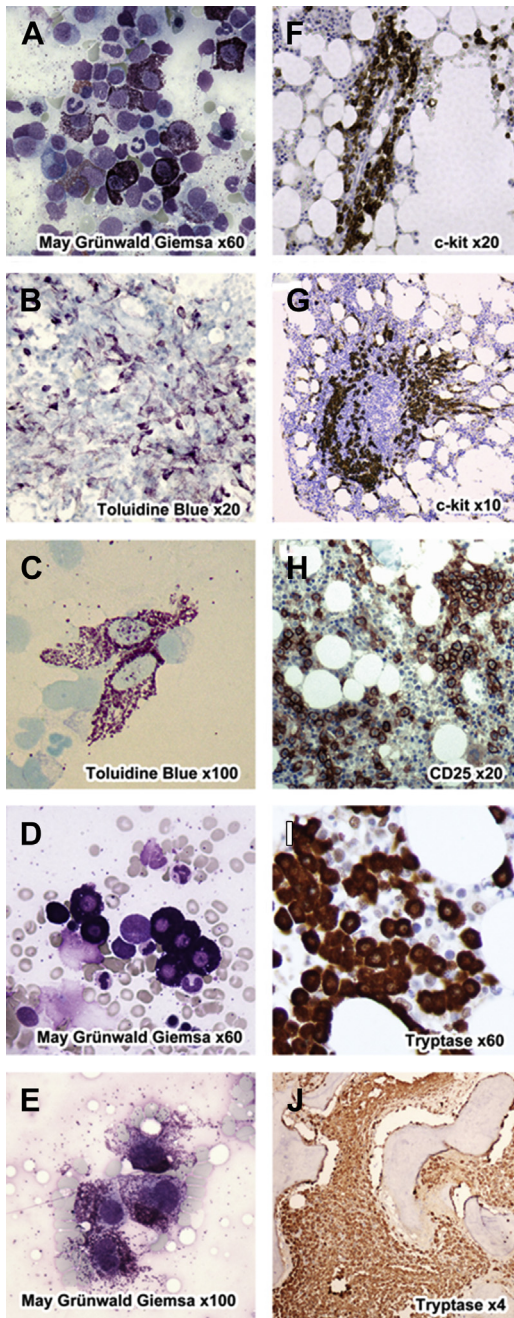


Fig. 2. Images of (A-E) BM smears and (F-J) BM sections from several SM patients. (A) High-power view showing fusiform MC together with round and oval MCs from a case of ISM. (B) Low-power view showing a marked increase of atypical MCs from a case of ASM. (C) High-power view showing 2 spindle-shaped MC. (D) High-power view showing round, fully granulated MCs from a case of WDSM. (E) High-power view showing morphologically poor-differentiated MC, some of which display 2 nuclei (promastocytes) from a case of

studies, the identification of MCs was based on their strong reactivity for CD117 in the absence of expression for CD38, CD34, and CD138, to discriminate MCs from CD34 HPC and CD117⁺ plasma cells; in turn, the coexpression of FcεRI and CD33 together with strong positivity for CD117 allows the discrimination between MCs and basophils.¹² More recently, it has been suggested that using a CD117^{high}/CD45^{low} combination could accurately identify BM MC^{12,13} (see Fig. 3).

Immunophenotypic Characteristics of Bone Marrow Mast Cells in Systemic Mastocytosis

Early studies on the immunophenotypic features of BM MCs in SM reported several aberrant phenotypes with a clear discrimination from normal and pathologic MCs.^{44–47} These early studies reported aberrant expression for CD2 and CD25 as a hallmark for SM, because both of these proteins were absent in normal/reactive BM MC, but expressed in most SM patients.^{9,48} From these 2 markers, CD25 has been described to be the most sensitive, specific, and stable, whereas positivity for CD2 may vary depending on the sensitivity of the fluorochrome-conjugated monoclonal antibody used in the study.¹² Also, in a recent publication,⁴⁹ it was shown that CD2 expression does not contribute to improve the diagnosis of SM when compared with aberrant CD25 expression alone.

Apart from the aberrant expression of CD2 and CD25, BM MCs from SM patients also display abnormally high expression of CD33⁴⁴ and of the CD2 ligand CD58.⁴⁵ Increased expression for activation markers such as CD69⁵⁰ or CD63⁵¹ and complement-associated molecules, such as CD11c, CD35,⁵² CD59, and/or CD88,^{52,53} are also commonly found in BM MCs from SM. In contrast, expression of CD117,⁴⁵ the CD71 transferrin receptor, and CD29 integrin is abnormally downregulated on BM MCs from these patients⁴⁸ (Table 1).

Aberrant expression of other molecules, such as CD30 (TNFR superfamily 8), seems not to be associated with the D816V KIT mutation itself. This receptor has been described recently to be widely expressed on BM MCs from SM patients and allows for the identification of a minor group of patients who typically lack expression of CD25/CD2 and activating exon 17 KIT mutations, WDSM patients, ultimately improving the overall diagnosis of the disease.^{4,54}

Coexistence of Normal and Pathologic Bone Marrow Mast Cells

Although the BM MC burden in ISM patients can be extremely low, the coexistence of normal and pathologic MCs in the same patient is frequent in specific disease categories (33% of ISMs— and 18% of ISM patients).⁷ Furthermore, the coexistence of normal and aberrant BM MC populations is usually restricted to SM patients with KIT mutation limited to the BM MC compartment,⁵⁵ and it should be considered a good prognosis factor because of its association with indolent forms of the disease.



ASM. (F) Low-power view showing a perivascular infiltrate of MCs from a case of ISM. (G) Low-power view showing a large lymphoid aggregate admixed with MCs from a case of ISM. (H) Low-power view showing CD25-positive MCs from a case of ISM. (I) High-power view showing round, fully granulated, tryptase-positive MCs from a case of WDSM. (J) Low-power view showing a dense, diffuse MC infiltration from a case of ASM. (From Alvarez-Twose I, Morgado JM, Sánchez-Muñoz L, et al. Current state of biology and diagnosis of clonal mast cell diseases in adults. *Int J Lab Hematol* 2012;34(5):452; with permission.)

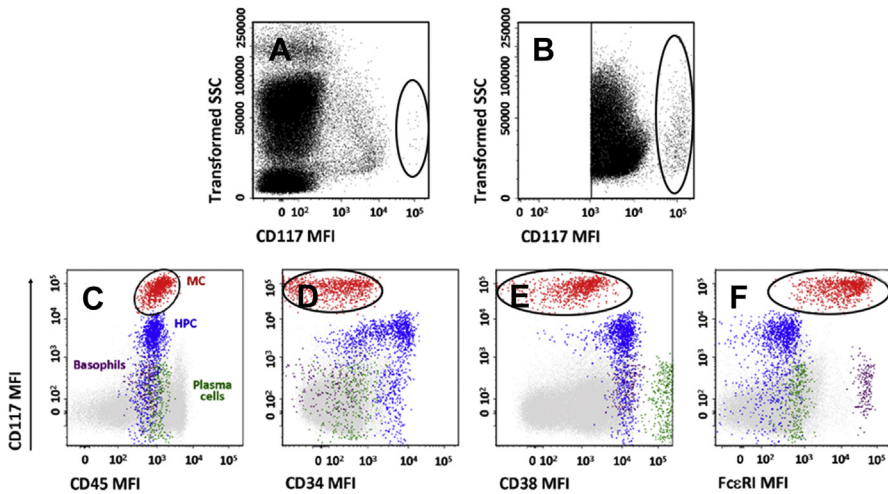


Fig. 3. The gating strategy proposed by REMA for the immunophenotypic identification and characterization of BM MCs by use of MFC. Whole BM cellularity (A) live gated to enrich on CD117⁺-nucleated cells corresponding to 3×10^6 total events (B) from an individual with a BM MC load of 0.009% is shown. (C–F) The gating strategy used for the identification of the BM MC population (red events) within the CD117⁺ cells gated in (B) and its phenotypic discrimination from CD34⁺ HPC (blue dots), plasma cells (green dots), and basophils (violet dots) coexisting in the same BM sample. (Adapted from Teodosio C, Mayado A, Sánchez-Muñoz L, et al. The immunophenotype of mast cells and its utility in the diagnostic work-up of systemic mastocytosis. *J Leukoc Biol* 2015;97(1):51; with permission.)

Mast Cell Immunophenotypical Expression of Different Types of Systemic Mastocytosis

Considering that SM is a group of heterogeneous diseases regarding both their clinical behavior and their prognosis,^{7,19,27,56–59} the differences in the immunophenotypic profiles of the distinct subtypes of SM were studied individually^{13,55}; in such an analysis, 3 different patterns were identified¹³ (Fig. 4).

Overall, the most frequently detected immunophenotypic pattern of BM MCs corresponds to a good-prognosis group of SM patients (ISM). These patients typically show a mature immunophenotype associated with aberrant expression of CD2 and CD25 and increased expression of MC activation-associated molecules.¹³ In line with this activation-related pattern, upregulation of molecules associated with MC degranulation (eg, the lysosomal glycoprotein CD63 and CD203c) is also observed in this subgroup of SM patients. Overall, these findings indicate that MCs from patients with ISM display an activated mature phenotype in the BM, associated with aberrant CD25 and CD2 expression (see Fig. 4).¹³ This activation-associated MC phenotype might result from the presence of the D816V KIT mutation, because this KIT mutation is shared by most (>95%) of these patients.¹⁴

In contrast to ISM, most BM MCs from WDSM patients typically do not carry the D816V KIT mutation in the absence of CD2 and CD25 expression, although approximately -third of cases are partially positive for CD25 or show dim expression of CD2 on its BM MC.^{13,49} BM MCs from WDSM patients display an overall phenotype similar to that observed for mature-resting, normal BM MCs (strong expression for CD117 and FcεRI).³⁸ Aberrant MC phenotypes from WDSM in most patients are

Table 1**Qualitative and semiquantitative patterns of expression of individual markers on normal, reactive, and systemic mastocytosis bone marrow mast cells**

Functional Group of Proteins	Surface Antigen	CD Code	Normal BM (% of Positive Cases)	Reactive BM (% of Positive Cases)	Overall SM (% of Positive Cases)
Cytokine receptor	IL-2R α	CD25	—	—	—/+ (93)
	IL-3R α	CD123	—	NR	—/+ (72)
	c-Kit	CD117	+++ (100)	+++ (100)	++/+++ (100)
Adhesion-related molecules	LFA-2	CD2	—	—	—/+ (72)
	Integrin alpha-L	CD11a	—/+ (20)	NR	—
	Integrin alpha-M	CD11 b	—/+ (50)	—/+ (50)	—/+ (50)
	Integrin alpha-X	CD11c	—/+ (71)	NR	+/++ (100)
	Integrin beta-2	CD18	+ (65)	NR	—/+ (44)
	Siglec-2	CD22	—/+ (60)	—/+ (50)	—/+ (96)
	Integrin beta-1	CD29	++ (100)	++ (100)	+ (100)
	Siglec-3	CD33	++/+++ (100)	++/+++ (100)	+++ (100)
	HPCA-1	CD34	—	—	—
	Pgp-1	CD44	++ (100)	++ (100)	++ (100)
	Integrin alpha-4	CD49 d	+/+++ (100)	NR	—/+ (80)
	Integrin alpha-5	CD49e	+ (100)	NR	—/+ (30)
	Integrin alpha-V	CD51	+ (100)	+	—/+ (45)
	ICAM-1	CD54	—/+ (75)	NR	++ (100)
Complement-related molecules	CR1	CD35	—	+	+ (100)
	Complement decay-accelerating factor	CD55	+ (100)	+ (100)	+/++ (100)
	Membrane attack complex inhibition factor	CD59	+ (100)	+ (100)	+/++ (100)
	C5aR	CD88	—/+ (18)	NR	—/+ (54)
Immunoglobulin receptors	Fc ϵ RI	NA	++/+++ (100)	++/+++ (100)	++/+++ (100)
	Fc γ RIIIB	CD16	—/+ (13)	—/+ (13)	—/+ (69)
	Fc γ RII	CD32	+ (100)	+ (100)	+/++ (100)
	Fc γ RI	CD64	—/+ (4)	—/+ (4)	—/+ (84)

MHC-related molecules	HLA-I	NA	++ (100)	++ (100)	+ / +++ (100)
	HLA-DR	NA	- / + (25)	- / + (25)	- / + (85)
Tetraspanins	CD9 antigen	CD9	+++ (100)	+++ (100)	+ / + / + / + (100)
	LAMP3	CD63	++ (100)	+ / + / + / + ^a (100)	+ / + / + / + (100)
TNF receptor family proteins	CD30 L receptor	CD30	–	NR	- / + (80)
	CD40 L receptor	CD40	- / + (65)	- / + (65)	- / + (65)
Mast cell proteases	Tryptase	NA	++ (100)	++ (100)	+ / + / + / + (100)
	Carboxypeptidase A	NA	++ (100)	++ (100)	+ / + / + / + (100)
Activation markers	Early activation antigen CD69	CD69	+ (100)	+ (100)	+ / + / + (100)
	E-NPP 3	CD203c	+ (100)	NR	+ / + / + (100)
Other	LCA	CD45	+ (100)	+ (100)	+ / + / + (100)
	Transferrin receptor protein 1	CD71	+ (100)	NR	- / + (38)
	Bcl-2	NA	- / + (94)	- / + (94)	- / + (87)

Abbreviations: NA, not applicable; NR, not reported; - / +, expressed in a subset of patients (percentage of positive cases); –, absent expression; +dim, dim positive expression; +, moderate positive expression; ++, positive expression; + / + / +, strong positive expression. Mixed symbols indicate variable reactivity, either among cases or among cells from the same individual.

^a Expression of CD63 is increased in BM MCs from myelodysplastic syndromes.

Data from Refs. [13,44–46,48,50–53,59,86](#)

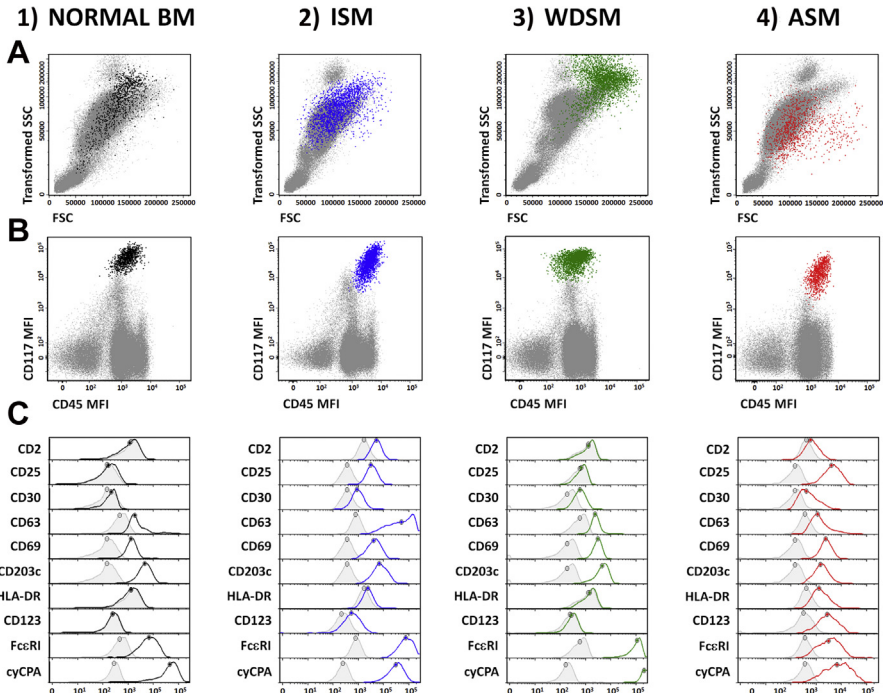


Fig. 4. (A, B) Representative bivariate dot plots and (C) histograms, illustrating the (A) light-scatter features and (C) immunophenotypical profiles of BM MCs from healthy donors (highlighted in black in A1, B1, and C1), ISM (highlighted in blue in A2, B2, and C2), WDSM (highlighted in green in A3, B3, and C3), and ASM (highlighted in red in A4, B4, and C4), identified on the basis of their reactivity for (B) CD45 and CD117. Baseline MC autofluorescence for each protein is indicated in the histograms in gray in C1–C4. cyCPA, cytoplasmic carboxypeptidase A. (From Teodosio C, Mayado A, Sánchez-Muñoz L, et al. The immunophenotype of mast cells and its utility in the diagnostic work-up of systemic mastocytosis. *J Leukoc Biol* 2015;97(1):55; with permission.)

restricted to expression of CD30 in the absence of CD25 high⁵⁴ and increased cytoplasmic contents in MC enzymes (eg, tryptase and CPA), which probably contribute to the typical hypergranulated, morphologic appearance of BM MCs in WDSM cases,^{4,15} and their abnormally increased size and internal complexity¹³ (see Fig. 4).

Oppositely, the poor-prognosis subvariants of the disease (ASM and MCL) typically display bright expression of CD25, frequently in the absence of CD2.^{13,49} This aberrant phenotype is commonly associated with increased expression of the CD123 and HLA-DR antigens, which are typically expressed by immature BM MC,^{38,60,61} together with abnormally low levels of cytoplasmic tryptase and CPA and decreased light-scatter (SSC and FSC) features (see Fig. 4).¹³ Overall, BM MCs from poor-prognosis variants of SM (ASM and MCL) display a relatively immature phenotype, potentially associated with an early blockade of MC maturation.

KIT Mutation Analysis in Mastocytosis

Most adults with mastocytosis carry the activating somatic KIT D816V mutation, whereas most cases of childhood-onset and familial mastocytosis would lack these

mutations. Nevertheless, the frequency of the D816V KIT mutation is highly variable in the literature, which could be due to several reasons:

- I. The relatively low number of cases included in some studies;
- II. The patient selection with predominance of aggressive versus indolent forms of the disease;
- III. The sensitivity of the methods used to assess the presence/absence of KIT mutations, particularly in cases with low MC burden;
- IV. The type of samples used for the identification of KIT mutations (eg, total BM samples versus highly purified BM MC).¹⁴

Standard Molecular Assays for Detection of KIT D816V

Mutation analysis based on genomic DNA (gDNA) or the expressed messenger RNA should be performed with caution, especially to avoid false-negative results in those BM samples with low MC numbers when low sensitive assays for detection of the KIT D816V mutation are used.¹⁴ Overall, in 6% of ISMs— other mutations involving exon 17 are described,¹⁴ such as D815K,⁶² D816F, E839K,²⁴ R815K,⁶² I817V,¹⁴ N819Y,⁶³ N822I,⁶⁴ D816Y,^{14,24} D816H,⁶⁵ insV815_I816I8, and D820G mutations.⁶⁶ In addition, other mutations of KIT out of exon 17 should be ruled out (eg, F522C, A533D, K509I, R634W, V560G, among others),^{67–74} as well as the pattern of inactivation of chromosome X by using HUMARA in BM MC,⁴ moreover in familial and WD mastocytosis.

Multiple molecular methods can be used to specifically detect the D816V KIT mutation in patients suspected of mastocytosis:

- I. Reverse transcriptase (RT)-polymerase chain reaction (PCR) assays plus restriction fragment length polymorphism,
- II. Nested RT-PCR followed by denaturing high-performance liquid chromatography of PCR amplicons,
- III. Peptide nucleic acid (PNA)-mediated PCR,
- IV. Allele specific PCR (ASO-PCR) and the quantitative variant of the method (ASO-qPCR) on DNA or RNA/complementary DNA (cDNA).⁷⁵

Other techniques include enrichment of MCs and other cell lineage from BM samples via laser microdissection, and both immunomagnetic^{76,77} and fluorescence-activated cell sorting^{12,14,76} of MCs followed by PNA-mediated PCR-clamping technique.^{55,62} These approaches showed the KIT mutation not only in BM MCs but also in other highly purified hematopoietic lineages in SM patients; 27% of ISM and almost all poor-prognosis SM patients carry the KIT mutation in other non-MC hematopoietic lineage, multilineal KIT mutation involvement,¹⁴ resulting the most powerful independent prognostic factor for progression of ISM to more aggressive forms of the disease.^{57,78} Multilineal KIT D816V mutation in SM can be detected in gDNA from CD34⁺ hematopoietic stem and precursor cells, eosinophils, monocytes, and maturing neutrophils, and, to a less extent also in T lymphocytes, in addition to BM MC.^{14,79} In order to evaluate the multilineal KIT pattern in SM, the REMA recommends performing the KIT mutation assays in at least one highly purified population (>97%) of myeloid cells (eg, BM monocytes and/or maturing neutrophils) and a population of lymphoid cells, apart from MC.^{10,57}

The D816V mutation in a peripheral blood (PB) sample from a patient with SM in the absence of detectable circulating MCs was described in 1995.²² Recently, several groups^{80–84} have confirmed the feasibility of detecting the KIT mutation in PB leukocytes using highly sensitive assays (eg, ASO-qPCR-assays). These approaches also

show that the mutation burden differs significantly among patients with different categories of the disease, but can be detected in PB leukocytes in nearly all adult patients with mastocytosis, including ISM and CM. In addition, the KIT D816V allele burden is of prognostic significance concerning survival and can be followed quantitatively during the natural course or during therapy and may be rapidly adopted as a valuable follow-up parameter in untreated and drug-treated patients with advanced SM.^{75,81}

Despite all the above, discrepant results regarding both the frequency of SM cases carrying the KIT D816V⁺ in PB and the prognostic impact of the KIT D816V allele burden have been published.^{81,83,85} These discrepant findings might be related to the different approaches and sensitivity of the techniques used to assess KIT mutation involvement in PB (analysis on gDNA vs cDNA), and also to SM cases with a very low KIT mutated MC burden. For these reasons, BM samples are still the preferred specimen to rule out the mutation of KIT, moreover when a very low BM MC burden is suspected.^{80,81,83}

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