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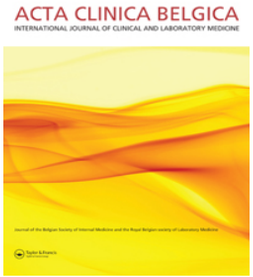

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Original Paper

Highly sensitive assays are mandatory for the differential diagnosis of patients presenting with symptoms of mast cell activation: diagnostic work-up of 38 patients

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Mastocytosis is a heterogeneous disease caused by excessive mast cell (MC) proliferation. Diagnosis of systemic mastocytosis (SM) is based on the presence of major and minor criteria defined by the World Health Organization. Symptoms of MC activation can also occur in patients without SM or without allergic or inflammatory disease. These MC activation syndromes (MCAS) can be divided into primary (monoclonal) MCAS (MMAS) vs. secondary and idiopathic MCAS. In this single center study, the diagnostic work-up of 38 patients with a clinical suspicion of SM and/or with elevated basic tryptase levels is presented. Clinical symptoms, biochemical parameters, results of bone marrow investigation, flow cytometric immunophenotyping, and molecular analysis were retrospectively reviewed. Twenty-three patients were found to have a monoclonal MC disorder of which 19 were diagnosed with SM and 4 with MMAS. In 13/19 SM patients, multifocal MC infiltrates in the bone marrow were found (major criterion), while in 6 the diagnosis was based on the presence of ≥3 minor criteria. Flow cytometric analysis of bone marrow showed CD25 expression of MCs in all patients with SM and MMAS (range: 0.002–0.3% of cells). In bone marrow, the *KIT* D816V mutation was detected in all SM patients but in only 2 patients with MMAS (range: 0.007–9% mutated cells). Basic tryptase elevation was demonstrated in 16/19 patients with SM but also in 9/19 patients without SM. Our study reveals the heterogeneity of primary MC disorders and the importance of sensitive assays in patients suspected of having SM.

Keywords: *KIT* D816V mutation, Mast cells, Monoclonal mast cell activation syndrome, Systemic mastocytosis

Introduction

Mast cells (MC) reside in the connective tissue of a variety of tissues and all vascularized organs. They are effector cells in allergic and inflammatory reactions. Degranulation of MC releases many pro-inflammatory mediators such as histamine and prostaglandin D2. These mediators cause typical symptoms of mast cell activation (MCA) such as skin symptoms (flushing, pruritus, urticaria, angioedema), gastro-intestinal symptoms (diarrhea, abdominal pain/cramping, gastroesopheagal reflux), bone pain, or fatigue.[1] MCA also often occurs in patients with a clonal proliferation of MC meeting World Health Organization (WHO) criteria for systemic mastocytosis (SM).[2,3] Hereby, the presence of multifocal, dense infiltrates of MC in bone marrow (BM) or other extracutaneous organs is considered as a major criterion. Minor criteria include serum basic tryptase (BT) level above 20 ng/mL, presence of atypical or spindle-shaped morphology in >25% of MC, detection of a *KIT*-mutation at codon 816 and expression of CD2 and/or CD25 by the MC. One major and one minor criterion or at least three minor criteria are sufficient for the diagnosis of SM.[2,3] SM is further classified into indolent and more aggressive forms. In indolent SM (ISM), the MC burden is low and skin lesions can be present. This is the most common variant of SM and patients with ISM have a

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life expectancy similar to that of the general population.[4] In aggressive SM (ASM), patients show organ dysfunction. In MC leukemia (MCL), the BM aspirate shows more than 20% MC and MC are often present in the peripheral blood smear. Systemic mastocytosis with an associated clonal hematological neoplasm (SM-AHN) meets both criteria for SM and an associated, clonal hematological non-mast cell lineage disorder.[3]

Some patients show symptoms of MCA but do not meet WHO criteria for SM. Recently, a working conference proposed a classification for these patients.[1] They define MCAS as the presence of suggestive clinical symptoms, an increase of serum BT level by at least 20% above baseline plus 2 ng/mL during or within 4 h after a symptomatic period and a response of clinical symptoms to histamine receptor blockers or MC targeting agents. They differentiate between idiopathic MC activation syndrome (MCAS), secondary MC activation syndrome (presence of an IgE mediated allergy), and primary (monoclonal) MC activation syndrome (MMAS). The latter is defined as MCAS with proven (mono)clonality of MC by expression of CD25 on MCs and/or detection of *KIT* D816V mutation.

Reactive hyperplasia of non-clonal MCs can be identified in tissue-biopsy specimens. People usually suffer from chronic inflammation, thrombosis, or cancer and may present with symptoms of MCA.[5,6]

To detect aberrant or clonal MCs, two highly sensitive techniques can be used, including flow cytometric immunophenotyping and molecular methods.[1,7] Flow cytometric detection of aberrant expression of CD2 and/or CD25 on MC is a reliable method to show monoclonality of MCs.[8,9] An allele-specific oligonucleotide real-time quantitative PCR (AS-qPCR) for *KIT* D816V is a sensitive method to identify mutation-positive cells in BM aspirates and plays an important role in diagnosis, disease monitoring and evaluation of prognosis in patients with SM.[10,11] Moreover, sensitive detection of *KIT* mutations and flow cytometric analysis have been shown to identify patients as having a clonal MC disorder while they had been previously diagnosed with idiopathic anaphylaxis.[12]

In this study, a work-up of 38 patients suspected of having SM is presented, in which the challenging differential diagnosis of MC pathology becomes clear, as well as the essential role of highly sensitive analytical methods.

Materials and methods

Patients

Thirty-eight patients were investigated because of symptoms suggestive of SM, associated with a BT level of >11 ng/mL in 32 of them and with skin mastocytosis in 15. Most patients had been referred to the outpatient department of Allergy and Clinical Immunology at UZ Leuven between October 2012 and November 2014. Clinical symptoms at the time of initial presentation, results of BM analysis, immunophenotypic, molecular, and biochemical studies were retrospectively reviewed in the patient files. The final diagnosis and classification were made according to the WHO criteria for SM and the diagnostic algorithm proposed by Valent et al.[1]

Bone marrow aspirate analysis

May-Grünwald-Giemsa-stained BM aspirate smears were carried out in all patients and were evaluated by two experts in bone marrow cytology. The percentage of MC of all nucleated cells was calculated by counting 300 hematological cells. Normal/reactive MC are usually loosely scattered throughout the sample, have round to oval shaped nuclei, clumped nuclear chromatin, low nuclear/cytoplasmic ratio and abundant cytoplasm with small granules. Atypical MC can be spindle-shaped and/or show hypogranularity, oval nuclei or immature features.[2,13,14]

Biopsies

Bone marrow biopsies were available in 37 patients. Samples were fixed in 10% buffered formalin, embedded in paraffin and stained with hematoxylin-eosin (HE), and Giemsa. For the confirmation of MC, biopsies were immunohistochemically stained for tryptase (Mast cell Tryptase Clone AA1; Dako Glostrup, Denmark) and CD117/*KIT* (Dako Glostrup, Denmark) and IL2R/CD25 (Clone 2A3, Becton Dickinson, Mountain View, USA).[15]

Immunophenotypic studies

A protocol for immunophenotypic analysis of MC was developed according to a proposal by Escribano et al.[16] BM samples were first passed several times through a 25-gauge needle to disaggregate BM particles and subsequently stained with CD117 APC, CD45 PerCP and CD25 PE antibodies (all from BD Biosciences, San Jose, USA). Samples were analyzed on a FACSCanto II flow cytometer (BD Biosciences, San Jose, USA) collecting $\geq 1 \times 10^6$ events. MC were identified by their high expression of CD117, intermediate CD45 expression and variable light-scattering characteristics using the FacsDiva Software (BD Biosciences San Jose, USA). Co-expression of CD25 identified aberrant MC. Cut-off value for positivity is 0.002% on all nucleated cells.

Molecular studies

KIT D816V mutation analysis was performed on all BM aspirates. *KIT* D816V was evaluated using an allele-specific real-time quantitative PCR assay, as reported.[10] Briefly, PCR was performed on a 7900 Real-Time PCR System (Applied Biosystems Massachusetts, USA). The detection limit is 0.005% mutated cells in a wildtype background.

Serum tryptase

Serum BT levels were measured by enzyme immunoassay using the ImmunoCAP 250 system (Phadia Uppsala, Sweden) with the normal value for BT below 11 ng/mL. In case of multiple consecutive measurements of serum BT, the highest level was taken.

Statistical analysis

A two-tailed unpaired *t*-test (Analyse-it®) was performed to determine the difference in % of CD25+ and *KIT* mutated MCs between SM and MMAS.

Results

In this group of 38 patients there were 1 child and 37 adults (median age: 50 years, range 6–75), 16 males, and 22 females (Table 1). Clinical symptoms at presentation consisted of anaphylaxis ($n = 18$), skin mastocytosis ($n = 15$), recurrent urticaria and/or flush ($n = 10$), gastrointestinal upset ($n = 7$) and multi-organ complaints ($n = 6$). Detailed information is represented in Table 2. BT levels were normal (<11 ng/mL) in 6 patients, between 11 and 20 ng/mL in 7 and >20 ng/mL in 25 patients.

Final diagnoses are summarized in Table 3. Nineteen patients were diagnosed with SM according to WHO criteria: 1 with ASM, 18 with ISM including 14 with skin involvement and 4 without skin involvement. Nineteen patients did not fulfill WHO diagnostic criteria for SM: 4 of the latter were diagnosed with MMAS and 3 with idiopathic MCAS. Four patients were found to have a different illness and were therefore categorized into 'other', although 3 out of 4 showed elevated BT. For another 8 patients, no final diagnosis could be given. No case was identified with MC leukemia.

The most frequent clinical symptom in the 23 patients with either SM or MMAS was anaphylaxis which was the presenting symptom in 13 patients (57%) (Table 2). In 9 of them (39%) severe, often recurrent, anaphylaxis to a hymenoptera sting was the first and often sole non-cutaneous manifestation of the monoclonal MC disorder. Other frequent symptoms in these 23 patients were skin mastocytosis (15/23, 65%), gastrointestinal symptoms (3/23, 13%), and recurrent urticaria or flush (2/23, 9%). Gastrointestinal biopsies were performed in 8/23 patients and showed an increased mucosal MC number in 5 of them (data not shown). However, because no CD25 staining was carried out, this could not be considered as a minor criterion for the diagnosis of SM. On top, 4/8 patients without a monoclonal MC disorder also showed an increased mucosal MC infiltration. Clinical symptoms of patients not classified as having SM or MMAS were more heterogeneous.

Table 4 shows the major and minor criteria in 38 patients. Of the 19 patients diagnosed with SM, 13 showed multifocal, dense infiltrates of MC in their BM biopsy (major criterion), while another 6 patients were diagnosed only by the positivity of at least three minor criteria. In all 19 patients with SM, aberrant expression of CD25 was found. A *KIT* D816V mutation and an elevated serum BT >20 ng/mL were present in 19/19 (100%) and 16/19 (84%) patients, respectively. In three patients with SM, focal spindle-shaped MC were seen, representing >25% of MCs. One patient with SM presented with osteolytic lesions and was therefore classified as ASM.

Flow cytometric analysis of the 19 patients with SM showed a mean of 0.043% of aberrant CD25 positive MCs on all nucleated cells (range: 0.003–0.30) (Graph 1). Patients with MMAS showed a mean of 0.01% CD25 positive MCs (range: 0.002–0.020). Statistical analysis showed no significant difference in CD25 positive MCs between patients with SM and patients with MMAS ($p = 0.32$).

Molecular data in the 19 SM patients revealed a mean of 0.79% *KIT* D816V mutated cells (range: 0.011–9.0). In one patient, mutational analysis was performed elsewhere and no exact percentage was determined. Only 2 of the 4 patients with MMAS had *KIT* D816V mutated MC. There was no statistical significant difference in the percentage of mutated MC between SM and MMAS patients ($p = 0.47$) (Graph 1).

Nineteen out of 38 patients did not fulfill the WHO criteria for SM. Of these, 4 patients were diagnosed with MMAS because clonality or aberrancy of MC was proved by mutational analysis ($n = 2$) and/or flow cytometry ($n = 4$). In the remaining 15 patients not meeting the SM criteria, no expression of CD25 and no *KIT* D816V mutation were found. Three of them were diagnosed with MCAS because of symptomatology of MC activation, a significant rise in tryptase level and a clinical response to therapy with MC-stabilizing agents.

A serum BT level exceeding 20 ng/mL was present in 18 of the 23 patients (78%) with a monoclonal MC disorder, with a higher frequency among the SM patients (16/19) than among the MMAS patients (2/4) of whom 1 subject had a level <11 ng/mL. Tryptase values of >20 ng/mL were on the other hand also found in half of the 15 patients (7/15) without monoclonal MC disorders. In the three patients with idiopathic MCAS, a significant increase of the tryptase level after an acute event was demonstrated as compared to the BT level which was <11 ng/mL in 2 of them.

Discussion

The clinical diagnosis of SM is very challenging because of the complexity, vagueness, and heterogeneity of many of its symptoms.[5] Therefore, in suspected cases, histological examination of BM biopsies is mandatory. In addition, because MC burden in SM may be very low, highly sensitive flow cytometric and AS-qPCR assays with detection limits as low as 2×10^{-3} and 5×10^{-3}, respectively, are required for detection of aberrant or clonal MCs in BM aspirates of these patients. In this retrospective study, we describe the diagnostic work-up of a series of 38 patients presenting with symptoms suggestive of SM: 39% of them had skin mastocytosis and 84% an increased BT level of >11 ng/mL.

MMAS and SM are clonal MC disorders. The latter can be further divided into ISM, SM-AHN, ASM, and MCL according to the WHO.[2,3] Nineteen out of 38 patients met

Table 1 Results of the diagnostic work-up in 38 patients presenting with MC activation symptoms. Case 1 to 23 are monoclonal MC disorders

Case	Sex	Age	Clinical symptoms	BM	BB	BT (ng/mL)	% D816V *KIT* mutated cells (on a wild-type background)	% CD25+ MC (on ANC)	Final diagnosis
1	M	63	UP + bone pain	nl	SM	34	0,420	0,038	ASM
2	M	50	anaphylaxis	nl	nl	28,4	0,031	0,021	ISM
3	F	41	UP + anaphylaxis	nl	#	22,6	0,079	0,005	ISM
4	M	53	anaphylaxis	*	SM	107	0,028	0,091	ISM
5	F	40	UP + gastro-intestinal + flush, vertigo, palpitations	nl	SM	22	0,210	0,036	ISM
6	M	56	UP + anaphylaxis	nl	SM	29	0,170	0,024	ISM
7	F	6	UP + gastro-intestinal	SM	ND	84	positive$	0,105	ISM
8	F	71	anaphylaxis	SM	SM	83	2,170	0,046	ISM
9	M	49	UP + anaphylaxis	nl	#	12,4	0,026	0,008	ISM
10	F	31	TMEP+ anaphylaxis	nl	SM	85	0,610	0,021	ISM
11	F	32	TMEP	nl	SM	32	0,100	0,008	ISM
12	M	31	anaphylaxis	nl	#	10,6	0,070	0,038	ISM
13	F	60	TMEP + anaphylaxis	nl	SM	52	0,300	0,008	ISM
14	M	48	UP	nl	SM	23,8	0,030	0,027	ISM
15	F	56	UP	nl	nl	33	9,000	0,026	ISM
16	F	26	UP + fatigue	nl	SM	56,4	0,280	0,012	ISM
17	F	42	UP	nl	SM	13	0,011	0,007	ISM
18	F	32	UP + flush	nl	SM	43	0,039	0,003	ISM
19	M	54	anaphylaxis	nl	SM	25	0,620	0,300	ISM
20	M	27	anaphylaxis	nl	nl	14	0,007	0,020	MMAS
21	F	38	papular rash + gastro-intestinal	nl	nl	23	0.000	0,002	MMAS
22	F	47	UP + anaphylaxis	nl	§	10,2	0,014	0,007	MMAS
23	M	54	anaphylaxis	nl	§	23	0.000	0,002	MMAS
24	M	50	anaphylaxis	nl	nl	10	0,000	0,000	MCAS
25	M	55	recurrent urticaria	nl	nl	22	0,000	0,000	MCAS
26	M	57	recurrent urticaria + anaphylaxis	nl	nl	4,6	0,000	0,000	MCAS
27	F	65	gastro-intestinal + fatigue	nl	nl	16,8	0,000	0,000	no final diagnosis
28	F	56	gastro-intestinal	nl	nl	21,2	0,000	0,000	no final diagnosis
29	M	46	recurrent urticaria	nl	nl	32	0,000	0,000	no final diagnosis
30	M	46	anaphylaxis	nl	nl	5,4	0,000	0,000	no final diagnosis
31	F	57	multiple non-specific symptoms	nl	nl	20,8	0,000	0,000	no final diagnosis
32	F	45	anaphylaxis	nl	nl	14,7	0,000	0,000	no final diagnosis
33	F	56	urticaria + anaphylaxis	nl	nl	17,7	0,000	0,000	no final diagnosis
34	F	61	gastro-intestinal + flush	nl	nl	17,1	0,000	0,000	no final diagnosis
35	M	28	gastro-intestinal	nl	nl	21,5	0,000	0,000	other (diffuse oxyuriasis)
36	F	75	throat swelling	nl	FL	28	0,000	0,000	other (FL)
37	F	45	Pruritus	nl	nl	20,4	0,000	0,000	other (Hogkin lymphoma)
38	F	52	gastro-intestinal + flush	nl	nl	5,9	0,000	0,000	other (lactose intolerance)

Note: Gastro-intestinal symptoms include abdominal pain, diarrhea and nausea; BM: bone marrow, BB: bone biopsy, nl: normal, BT: basic tryptase, UP: urticaria pigmentosa
*elevated MC count but not spindle shaped
§aberrant MCs <25%
#>25% aberrant MCs in infiltrate but major criterion not fulfilled, FL: Follicular lymphoma
$exact value not specified

the WHO criteria for SM and were definitively diagnosed as having ISM or ASM. Another 4 patients fulfilling only 2 minor criteria were categorized as MMAS, making a total of 23 patients with a monoclonal MC disorder which were further reviewed.

The presence of increased MC numbers in extracutaneous organs is not considered diagnostic for SM, whereas a multifocal dense infiltrate of spindle-shaped MC in a BM biopsy is a major criterion and the presence of MCs with atypical morphology in >25% of them is a minor criterion.[14] Of the 19 patients with SM, the BM biopsy (not available in 1 patient) showed abnormal MC distribution or spindle shaped MC in 89% (16/18), whereas in only 16% (3/19) aberrant MC and/or an elevated MC count

Table 2 Presenting symptoms in 38 patients

	Monoclonal MC disorder	Non-monocloncal MC disorder	All patients
	(*N* = 23)	(*N* = 15)	(*N* = 38)
	(%)	(%)	(%)
Skin mastocytosis	15 (65)	0	15 (39)
Urticaria pigmentosa (UP)	12 (52)	0	12 (32)
Teleangiectasia macularis eruptiva perstans (TMEP)	3 (13)	0	3 (8)
Recurrent urticaria or flush	2 (9)	6 (0,4)	8 (21)
Anaphylaxis (often recurrent)	13 (57)	5 (33)	18 (47)
Hymenoptera sting	9 (39)	0	9 (24)
Drug induced	2 (9)	1 (7)	3 (8)
Idiopathic (e.g. radiocontrast, postprandial)	3 (13)	4 (27)	7 (18)
Abdominal pain/bloating, nausea, diarrhea	3 (13)	5 (33)	8 (21)
Multi-organ symptoms (pruritis, throat swelling, palpitations, vertigo)	2 (9)	4 (27)	6 (16)

Table 3 Final diagnosis of 38 patients

	Diagnosis	Number of patients
Monoclonal mast cell disorders (***n*** = 23)	Aggressive systemic mastocytosis (ASM)	1*
	Indolent systemic mastocytosis (ISM)	18*
	Monoclonal mast cell activation syndrome (MMAS)	4
Non-monoclonal disorders (***n*** = 15)	Idiopathic mast cell activation syndrome (MCAS)	3
	Other	
	Parasitosis (diffuse oxyuriasis)	1
	Lactose intolerance and rosacea	1
	Follicular lymphoma	1
	Hodgkin lymphoma	1
	No final diagnosis	8

*Patients with systemic mastocytosis according to WHO criteria.

were seen in the BM smear, stressing the superiority of BM biopsies over an aspirate smear for the diagnosis of SM. This was also found by Sperr et al. who studied 69 patients with SM of which the majority showed a BM aspirate MC count of <5%.[13] However, a BM aspirate remains necessary to exclude MCL and to diagnose SM-AHN.

In 32% (6/19) of our cases, the diagnosis of SM was based only on the presence of at least 3 minor WHO criteria. The presence of a *KIT*D816V mutation is one of these minor WHO criteria. Assays with a low limit of detection such as AS-qPCR have demonstrated a *KIT*D816V mutation in > 90% of SM cases, whereas less sensitive methods such as Sanger sequencing have resulted in false-negativity in the presence of a low MC burden. Other mutations of *KIT* have also been reported in SM but are less frequent.[14,17,18] In our study all of the 19 patients with SM were positive for the *KIT* D816V mutation and the assay could detect as little as 0,007% positive cells. Moreover, in 2 out of the 4 MMAS patients, MC clonality was also proven by this high sensitive mutational analysis.

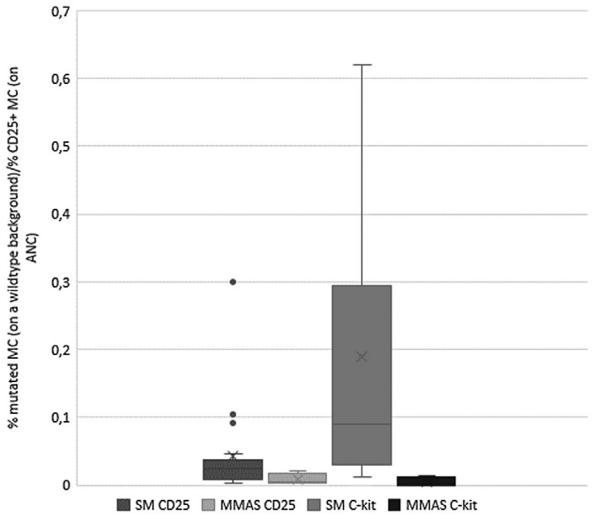
The presence of aberrant CD2 and/or CD25 expression on MCs by flow cytometric analysis is also a minor criterion for SM. As CD2 and/or CD25 expression are not unique to MC, gating on CD117 is required to identify MCs. Because of the low frequency of MC in BM, at least 10^6 events need to be acquired.[9] Only CD25 expression was studied because addition of CD2 seems to have limited value.[19,20] Morgado et al. showed a similar sensitivity but a slightly higher specificity when CD25 expression alone was measured in comparison with the inclusion of CD2.[19,20] In our study, in all patients with ISM as well as in all those with ASM and MMAS, CD25 positive MCs could be detected. Besides, in 2 out of the 4 MMAS patients, aberrancy of MC was detected by flow cytometric analysis but not by mutational analysis, thereby highlighting the complementary importance of the immunophenotypic assay. The consideration is particularly important in patients with recurrent sting or idiopathic anaphylaxis having an almost normal BT level as was the case in 2 of the MMAS patients. The high sensitivity of the assay was also shown in a study of Pozdnyakova et al. in which one patient could be diagnosed with MMAS, only because of this CD25 flow cytometric analysis on BM cells.[9]

Dosage of serum BT, the main secretory granule-derived proteinase in MC, is an important diagnostic parameter and a level of >20 ng/mL is another minor criterion for the diagnosis of ISM. In most patients with ISM, serum BT levels indeed exceed 20 ng/mL.[14] This was also the case in our study where only 3 patients (17%) with ISM did not fulfill this minor criterion. Nevertheless, an elevated BT is not a very specific parameter because also other diseases can be associated with an increased serum BT including acute myeloid leukemia, myelodysplastic syndromes, hypereosinophilic syndrome associated with the *FIP1L1-PDGFRA* mutation and probably many other yet unknown disorders.[6] In our study 73% (11/15) of patients with non-monoclonal MC disorders had a serum BT >11 ng/mL of whom 7 exceeded 20 ng/mL. We might speculate that increased BT levels in these patients could be caused by a reactive MC hyperplasia, e.g. in association with a lymphoma (n = 2) or at the intestinal level, where

Table 4 Major and minor criteria in 38 patients presenting with MCA symptoms. The gray shaded area represent monoclonal MC disorders.

	All	Patients meeting SM criteria (*n* = 19)			Patients not meeting SM criteria (*n* = 19)		
	n = 38	ASM (*n* = 1)	ISM (*n* = 18)	MMAS (*n* = 4)	MCAS (*n* = 3)	Other (*n* = 4)	No final diagnosis (*n* = 8)
	***N* (%)**	***N***	***N***	***N***	***N***	***N***	***N***
Major criterion	13/37* (35)	1	12/17*	0	0	0	0
Minor criteria							
Atypical morphology	5 (13)	0	5	0	0	0	0
Aberrant CD25⁺ phenotype	23 (61)	1	18	4	0	0	0
KIT D816V mutation	21 (55)	1	18	2	0	0	0
Serum tryptase >20 ng/mL	25 (66)	1	15	2	1	3	3
		Monoclonal MC disorders (***n*** = 23)			Non-monoclonal MC disorders (***n*** = 15)		

Abbreviations: ASM: aggressive systemic mastocytosis, ISM: indolent systemic mastocytosis, MMAS: monoclonal mast cell activation syndrome, MCAS: idiopathic mast cell activation syndrome, MC: mast cells
*In one patient no BM biopsy was obtained.



Graph 1 Box and whisker plot of CD25+ MC and *D816V KIT mutated* MC in SM (*n* = 19) and MMAS (*n* = 4).
Notes: 2 outliers (9 and 2.17% *D816V KIT mutated* MC of SM patients) are not visualized; in one SM patient the quantitation of the *p.D816V KIT* mutation was not available. (p = 0.32 between SM and MMAS for CD25+, p = 0.47 between SM and MMAS for *c-kit*.)

increased MC infiltration was shown in 4 of the 8 patients who had intestinal biopsies. Therefore, an increased serum BT level cannot be regarded as a specific marker for SM.[14] On the other hand, a normal serum BT level does not exclude SM either, as was recently proven in patients with sting anaphylaxis.[21] Idiopathic MCAS, an entity described by Valent et al. was diagnosed in 3 of these 15 patients.[1] However, the number might have been much higher if tryptase after an acute event had been determined in all 15 patients instead of in only 3 of them. Nevertheless, MCAS and reactive MC hyperplasia are entities that are not well understood.

According to the literature, symptoms in SM vary from mild with no need for therapy to life threatening.[22,23] Many patients with SM suffer from multiple non-specific symptoms which only, when associated with urticarial pigmentosa, point to the presence of SM. In contrast, in 12 of our patients with SM or MMAS, severe anaphylaxis was the first and only manifestation of the disease. Recurrent anaphylaxis, particularly when associated with urticarial pigmentosa or with BT levels >11 ng/mL are indications for a BM biopsy. In our study, 13 of the 18 patients (72%), who presented with anaphylaxis, were diagnosed with either ISM or MMAS. Nine out of the 23 patients (39%) with a monoclonal MC disorder had experienced a severe sting anaphylaxis which is in concordance with the study of Gülen et al.[24] The importance of BM biopsies in patients with severe sting anaphylaxis has been recently stressed by the study of Zanotti who showed a 77% prevalence of monoclonal MC disease despite normal BT levels in 22 patients with anaphylactic shock after a hymenoptera sting without skin lesions.[21] On the other hand, if serum BT is >20 ng/mL, a positive CD25 flow cytometry on the BM aspirate and a positive *KIT* mutation analysis are sufficient to diagnose a monoclonal MC disease. Thus, strictly there is no need to perform a BM biopsy in all such cases.

To conclude, MC disorders are very heterogeneous, challenging to diagnose and a careful diagnostic work-up is essential since each disorder has different prognosis and treatment options. Although only a limited number of patients were included in this study, our data show the importance of the combination of both flow cytometric and molecular assays with very low detection thresholds to correctly diagnose patients suspected of having SM. For 6 of our 19 patients (32%) with SM, the diagnosis was based entirely on minor criteria, which is in agreement with literature reports that the major criterion is negative in about 20% of SM patients.[25,26] In addition, diagnosis of MMAS, which is reported with an increasing frequency,

particularly in patients with severe sting anaphylaxis can only be made using assays that allow quantification of as little as 2×10^{-3} CD25-positive MCs and 5×10^{-3} *KIT* D816V mutation-positive cells.

Contributors

BVDP collected the data and wrote the manuscript. AMK participated in the study design and reviewed clinical data. EDB collected data. BD, EL and PV performed the molecular studies. TT and GDH provided the anatomopathological data. NB designed and coordinated the study. All authors read and approved the manuscript.

Conflict of interest

No potential conflict of interest was reported by the authors.

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