

Diagnostic and Subdiagnostic Accumulation of Mast Cells in the Bone Marrow of Patients with Anaphylaxis: Monoclonal Mast Cell Activation Syndrome

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Key Words

Anaphylaxis • CD2 • CD25 • *KIT* mutation • Mastocytosis • Tryptase

Abstract

Background: Patients with mastocytosis may suffer from severe hypotension after wasp or bee stings. In these patients, no specific IgE is detectable, but they usually have skin lesions and an elevated serum tryptase level. **Methods:** We report on 6 patients who were referred to our department because of severe hypotension following bee or wasp stings without cutaneous lesions. **Results:** In 3 patients, the baseline serum tryptase level was elevated (26, 36, and 67 ng/ml, respectively), and investigation of their bone marrow revealed systemic mastocytosis (SM). In the remaining 3 patients, serum tryptase levels were <20 ng/ml, and bone marrow histology and tryptase immunohistochemistry did not reveal diagnostic mast cell infiltrates. However, in 1 patient, three minor SM criteria were demonstrable leading to the diagnosis SM, and in the 2nd patient, two minor SM criteria, including an aberrant mast cell phenotype, were found. In the 3rd patient, no minor SM criteria were detected. **Conclusions:** All patients with unexplained hypotension after hymenoptera stings should undergo a thorough investigation for major and minor SM criteria regardless of the tryptase

level or presence of skin lesions, in order to diagnose or exclude SM or a related subdiagnostic condition (1 or 2 minor SM criteria) tentatively termed monoclonal mast cell activation syndrome.

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Introduction

Mastocytosis is a heterogeneous disease characterized by abnormal accumulation of mast cells (MC) in one or more organs [1–4]. Cutaneous mastocytosis (CM) frequently develops in infancy and has a favorable prognosis with frequent spontaneous remission before, at, or shortly after puberty [5]. Systemic mastocytosis (SM) develops at any age and is usually characterized by multifocal infiltrates of MC in one or more extracutaneous organs (major SM criterion) with or without skin involvement [1–4, 6, 7]. In the vast majority of these patients, the bone marrow is affected. In most SM patients, the somatic *KIT* mutation 2468A→T (D816V) is detectable [8–11]. Other minor criteria of SM include cytomorphological features of MC (spindle shape), an increased serum tryptase level (>20 ng/ml), and expression of CD2 and/or CD25 in *KIT*-positive bone marrow MC [12–18]. If at least one major and one minor or at least three minor SM criteria are ful-

filled, the diagnosis SM is established [19, 20]. Based on the organ system(s) involved, clinical course, and the presence or absence of hematologic abnormalities (or a hematologic disease), SM can be divided into four distinct categories: indolent SM, SM with an associated hematologic clonal non-mast cell lineage disease, aggressive SM, and MC leukemia [19, 20].

In a smaller group of patients with SM, typical skin lesions of mastocytosis are absent. In patients with aggressive SM and MC leukemia, the absence of skin lesions is a pathognomonic feature [19, 20]. However, not all SM patients in whom skin lesions are absent have aggressive disease. Likewise, patients may have isolated bone marrow mastocytosis, a special disease variant characterized by the absence of skin lesions and a low serum tryptase level [19, 21]. Most of these patients may not be diagnosed but overlooked unless their clinical symptoms (unexplained severe hypotension and osteoporosis, for example) raise the suspicion of an underlying MC disease.

In patients with mastocytosis, clinical problems often occur from the release of MC-derived vasoactive mediators [22–28]. Typically, patients with severe unexplained (allergy-test-negative) hypotension after wasp or bee stings are diagnosed to have (so far unrecognized) mastocytosis. In some of these cases, fatal anaphylactoid reactions have been reported [23–28]. Therefore, it is of importance to consider SM as a differential diagnosis in these patients, and thus to apply SM criteria.

In the current article, we report on 6 patients in whom hypotension occurred in response to insect stings, but neither CM nor specific IgE were detectable. Application of SM criteria revealed SM in 4 of these 6 patients, and two minor SM criteria in 1 patient. We recommend to call this condition monoclonal MC activation syndrome (MMAS) and to treat these patients in the same way as those with SM plus anaphylaxis.

Patients and Methods

Patients

Six patients (1 female and 5 males) with unexplained severe hypotension after hymenoptera stings were referred to our department between January 2004 and December 2005. The patients' characteristics are shown in table 1. All patients had been seen by an allergologist and dermatologist prior to admission. Clinical and laboratory investigations for specific IgE including RAST (bee and wasp specific; detection limit: 0.35 kUA/l; Pharmacia CAP FEIA, Uppsala, Sweden) and skin prick tests were negative in all patients. In 1 patient, a single skin macula was identified and found to contain slightly elevated numbers of MC by histology (table 1). Otherwise, no skin lesions were detectable at

the time of referral. However, in 1 patient, the case history revealed childhood urticaria pigmentosa, which disappeared before puberty. No patient suffered from an endocrinologic, neurologic, or cardiovascular disorder. In all patients, hypotensive episodes were severe requiring hospitalization. One patient (patient 3 in table 1) was referred as 'sudden death survivor' by the cardiologist. At the time of referral, no specific clinical symptoms indicative of anaphylaxis or SM were noted.

Bone Marrow Histology and Immunohistochemistry

Bone marrow was obtained from the iliac crest after informed consent was given. Specimens were fixed in ethanol-formaldehyde solution, followed by overnight decalcification in EDTA and embedding in paraffin. Immunohistochemistry was performed on 2- μ m sections using the avidin-biotin immunoperoxidase staining technique as described [13, 29, 30]. Endogenous peroxidase was blocked by methanol and H₂O₂. Antibodies were diluted in Tris-buffered saline (pH 7.5) containing 1% bovine serum albumin, and applied at room temperature. The antibodies used and the staining technique are depicted in table 2. After each step, slides were rinsed in Tris-buffered saline. After incubation with antibodies, slides were washed and incubated with biotinylated horse anti-mouse or goat anti-rabbit IgG for 30 min. Sections were washed and exposed to avidin and biotinylated peroxidase complex for 30 min. 3-Amino-9-ethyl-carabazole was used as chromogen. Slides were counterstained in Mayer's hemalaun.

Evaluation of Bone Marrow Smears – Cytology

In all patients, Wright-Giemsa-stained bone marrow smears were carefully examined for the presence (percentage) of MC and their morphology using published guidelines for morphological grading [15]. In addition, the non-MC compartment of the bone marrow smear was carefully reviewed in each case.

Flow-Cytometric Investigation of Bone Marrow MC

Bone marrow MC were analyzed for expression of disease-related cell surface antigens by multicolor flow cytometry as described previously [18, 30–32]. In brief, heparinized bone marrow samples were incubated with combinations of monoclonal antibodies (mAbs) [32] and then subjected to erythrocyte lysis and fixation by exposure to FACS lysing solution (Becton Dickinson, San Jose, Calif., USA). The FITC-labeled mAbs RPA-2.10 (CD2), 2A3 (CD25), and 581 (CD34), the PerCP-labeled mAb 2D1 (CD45), and the PE-labeled mAb RPA-2.10 (CD2) were purchased from Becton Dickinson. The APC-labeled mAb 104D2D1 (CD117) was purchased from Beckman Coulter/Immunotech (Marseille, France). After immunostaining, cells were analyzed on a FACS-Calibur (Becton Dickinson) as described [31, 32].

Analysis of Mutations at Codon 816 of KIT

Bone marrow mononuclear cells were isolated by Ficoll separation and subjected to RNA isolation using RNeasy as described [11]. mRNA was analyzed for the presence of the KIT mutation 2468A→T (D816V) by reverse transcriptase polymerase chain reaction and restriction fragment length polymorphism analysis using *Hinf*I digestion according to a previously published technique [11]. The human MC leukemia cell line HMC-1, known to display the KIT mutation D816V [33], served as a positive control.

Table 1. Patient characteristics and diagnoses

No.	Age years	Sex	Final diagnosis	Hypotension to (trigger)	Skin- lesions	Serum tryptase ng/ml	Spindle shaped MC in BM smears %	Tryptase+ BM MC infiltrates %	KIT D816V in BM MNC	Phenotype of KIT+ MC	
										CD2	CD25
1	33	m	SM	ant, wasp	no	67	>25	5	+	+	+
2	33	m	SM	bee, wasp	no ¹	36	>25	5	+	NT	+
3	48	m	SM	bee	no	11	>25	<5	+	+	+
4	50	m	SM	wasp	no	26	>25	<1	–	–	+
5	39	f	MMAS	wasp	no ²	12	>25	<1	–	+	–
6	65	m	– ³	wasp	no	18	<25	<1	–	–	–

BM = Bone marrow; MNC = mononuclear cells; NT = not tested.

¹ In this patient, one single macule was detected and found to contain elevated dermal mast cell numbers.

² In this patient, urticaria pigmentosa was diagnosed in early childhood but then disappeared before puberty.

³ In the absence of any signs of MC involvement, this patient was diagnosed as having unexplained hypotension.

Table 2. Specification of antibodies used for immunohistochemistry

Antibody	CD	Reactive antigen	Immunoglob- ulin type	Antibody dilution	Antibody source
4C9	CD25	IL-2R α	IgG2b	1:50	NC
QBEND10	CD34	HPCA-1	IgG1	1:50	IT
Anti-kit	CD117	c-kit/SCFR	polyclonal	1:500	D
G3	NYC	Tryptase	IgG1	1:5,000	C

IL-2R α = Interleukin-2 receptor α ; HPCA-1 = hematopoietic progenitor cell antigen-1; SCFR = stem cell factor receptor; NYC = not yet clustered; NC = Novocastra, New Castle, UK; IT = Immunotech, Marseille, France; D = Dako, Glostrup, Denmark; C = Chemicon, Temcula, Calif., USA.

Analysis of Serum Tryptase Concentrations

Serum total tryptase levels were determined by a commercial fluoroenzyme immunoassay purchased from Pharmacia Diagnostics (Uppsala, Sweden). The detection limit of the test was 1 ng/ml.

Results

Clinical Checkpoints and Decision to Investigate the Bone Marrow

The leading clinical symptom and primary decision point was severe hypotension following a bee or wasp sting without a positive allergy test (RAST). Based on these findings, the basal serum tryptase level was measured. In those with basal tryptase >20 ng/ml (n = 3), a bone marrow investigation was performed. In those with

a tryptase level <20 ng/ml, all laboratory and clinical parameters were reevaluated. In 1 of these patients, careful reassessment of the case history (the patient asked her parents for skin diseases in infancy) revealed urticaria pigmentosa in early childhood, which later resolved spontaneously. Finally, we decided to perform a bone marrow investigation in all 6 patients regardless of the serum tryptase level. The major reason for the decision to investigate the bone marrow (secondary decision point) was the typical trigger (insect stings).

Bone Marrow Histology Findings

In all patients, tryptase immunohistochemistry was performed. In 2 of the 3 patients with elevated serum tryptase, the bone marrow histology and tryptase stain revealed typical small-sized compact infiltrates com-

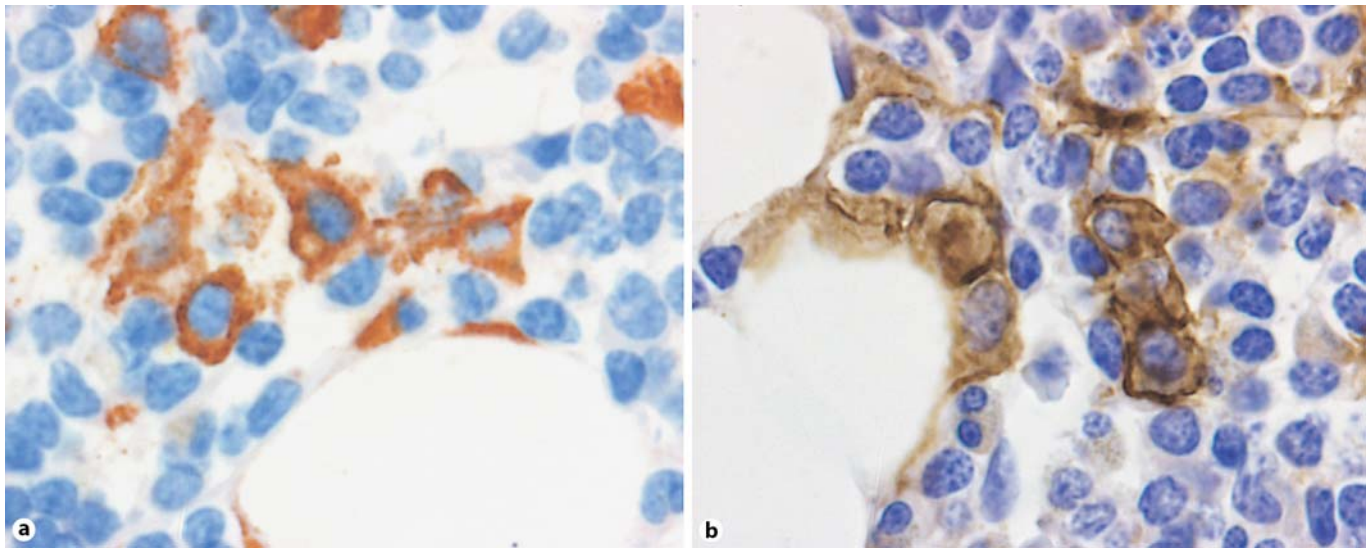


Fig. 1. Subdiagnostic aggregates of MC in a patient with anaphylaxis. MC were visualized by immunohistochemistry using paraffin-embedded bone marrow sections and antibodies against tryptase (**a**) or KIT/CD117 (**b**). MC were found to form small aggregates, whereas larger (diagnostic) infiltrates were not detectable. Nevertheless, the diagnosis of SM could be established in this patient based on the presence of three minor SM criteria.

posed of spindle-shaped MC (major SM criterion). As assessed by immunohistochemistry, these cells were also found to stain positive for CD117/KIT and CD25. In 1 patient with elevated tryptase (26 ng/ml), no diagnostic MC infiltrates were detected, although MC showed an abnormal morphology (fig. 1). In the 3 patients with serum tryptase levels <20 ng/ml, no compact MC infiltrates were detected by histology and tryptase staining.

Morphology of MC in Bone Marrow Smears

In all 3 patients with compact MC infiltrates, the majority of MC in the bone marrow smears exhibited clear cell atypia with prominent cytoplasmic extensions, oval nuclei, and a hypogranulated cytoplasm, thus resembling atypical MC type I according to established criteria [15]. Many of these MC were found to be spindle-shaped cells. In 2 of the 3 patients without histological evidence of SM (patients 4 and 5), MC were found to exhibit a spindle shape morphology consistent with atypical MC type I (fig. 1), whereas in 1 patient, MC did not show characteristic cell atypia, but were found to resemble normal tissue MC with a round central nucleus and a well-granulated cytoplasm. No increase in the number of MC in the bone marrow smear was found in this patient. Atypical MC type II or metachromatic blasts [15] were not recorded in any of the patients examined.

Phenotype of Mast Cells – Flow-Cytometric Results

In all 3 patients with diagnostic bone marrow MC infiltrates, MC were found to stain positive for CD2 and CD25 by flow cytometry (table 1). In 2 patients without diagnostic MC infiltrates, MC were also found to stain positive for CD2 and/or CD25. However, in 1 patient, MC were clearly found to lack CD2 and CD25. Interestingly, in this patient, MC were mature round cells and were negative for KIT D816V. There was also no increase in the number of MC detected by flow cytometry in this patient.

Results from KIT Mutation Analysis

In all 3 patients with histological evidence of SM, the KIT point mutation D816V was found in bone marrow mononuclear cells (table 1). By contrast, in the other 3 patients, this mutation was not detectable.

Final Diagnosis

In 4 patients, the final diagnosis was SM by WHO criteria. In 3 patients, both major and minor criteria of SM were detected, whereas in 1 patient, only three minor criteria, but no major criterion, were found. In 1 patient, indirect signs of a population of monoclonal MC as well as signs of MC activation were found. However, only two minor SM criteria (presence of atypical MC and expres-

sion of CD25 on MC) could be substantiated, so that we were unable to diagnose SM. This patient was tentatively diagnosed as having MMAS. In 1 patient, no SM criterion could be detected, and we were unable to link clinical symptoms to the MC lineage or to MC activation with any certainty (normal serum tryptase). Therefore, the final diagnosis in this patient was unexplained hypotension.

Clinical Course and Recommended Therapy

All patients including the one with unexplained hypotension were advised to avoid all situations or potential triggers that could provoke an event (especially bee and wasp stings). In all patients in whom MC activation was found, including the patient with MMAS, prophylactic H1 and H2 receptor antagonists were administered. In addition, these patients were advised to carry epinephrine self-injectors and to use them on demand, i.e. in case of severe anaphylaxis. All patients undergo regular (yearly) follow-up investigations in our department including serum tryptase measurements.

Discussion

MC activation with consecutive release of vasoactive mediators often represents a major clinical problem in patients with SM [22–28]. In most cases, the disease is known when such mediator-related symptoms occur. In a smaller group of patients, however, no skin lesions are present, and the existence of SM is not known at the time of first symptoms. Such cases can represent a challenge for the physician, especially when basal serum tryptase levels are normal. We report on 6 patients without skin lesions in whom severe hypotension had occurred after bee or wasp stings. In 3 patients, increased serum tryptase was found and consecutive examination of the bone marrow revealed SM. In 1 patient, the SM could be diagnosed despite a normal serum tryptase level, as in this patient three minor SM criteria were fulfilled. In another patient, only two minor SM criteria were detected – this patient was diagnosed to have MMAS.

Severe hypotension can develop in any category of SM and can represent a serious clinical problem [22–28]. In those patients who do not show typical skin lesions, these symptoms may be confused with allergies, endocrinologic or cardiac disorders, or other internal diseases. Therefore, it is important to examine these patients in a stepwise fashion using an appropriate diagnostic algorithm [27, 28]. In our study, the patients had already been ex-

amined for the presence of a hymenoptera allergy, or an endocrinologic or cardiac disease before being referred. In addition, most patients had already undergone serum tryptase examination before they were seen at our department. In those with elevated baseline tryptase, the investigation of the bone marrow appears to be a straightforward diagnostic approach to exclude or confirm the presence of SM. However, based on our results, several of these patients may have SM even if the serum tryptase level is normal. Therefore, it may be preferable to recommend a bone marrow study in all patients with unexplained severe hypotension after hymenoptera stings in whom no allergy can be documented. Thus, severe hypotension after hymenoptera stings may be regarded rather as a typical finding in patients with SM [23–26, 32].

SM is defined by major and minor SM criteria. If at least one major and one minor or three minor SM criteria are fulfilled, the diagnosis SM can be established [19, 20]. In 2 of our patients, histological evidence of SM (major SM criterion) was provided by tryptase immunostaining of bone marrow sections. In these patients, MC infiltrates were small in size but consisted of at least 15 MC per aggregate. In addition, these MC were found to be spindle-shaped cells and to coexpress CD25 with KIT (minor SM criterion) so that the diagnosis SM could be established without further testing.

In 4 of the 6 patients, no diagnostic MC infiltrates were detected by histology and immunohistochemistry. In these patients, minor SM criteria were applied. Three minor SM criteria were found in 2 of these patients consistent with the diagnosis SM. In 1 patient, no minor SM criterion was found – this patient was diagnosed to suffer from unexplained hypotension.

In 1 patient, two minor SM criteria were found, namely an abnormal morphology of MC in Wright-Giemsa-stained bone marrow smears and abnormal expression of CD25 on MC. In this patient, the final diagnosis SM could not be established although (indirect) signs of a monoclonal population of MC were present. In this regard, it is noteworthy that atypical MC type I (spindle-shaped MC), which were found in our patients, are usually confined to SM. In addition, expression of CD25 on MC is considered to be a specific feature for SM and thus a clear – albeit indirect – sign of MC monoclonality. Based on our findings, we decided to term the condition MMAS. The same condition, i.e. only two minor SM criteria, has recently been described by Akin et al. [34]. However, in their study, bone marrow MC were found to exhibit KIT D816V as direct sign of monoclonality of MC [34]. Therefore, this condition may be judged as closely related to

SM, and the patients be regarded as having a (potential) prephase of SM.

The *KIT* mutation D816V is a pathognomonic sign of SM [8–11]. This mutation is highly associated with an aberrant MC phenotype, i.e. expression of CD25 with or without CD2 [19, 20]. Most of our patients were also found to display *KIT* D816V. However, in the patient with MMAS, the mutation was not detectable. This negative result may have several explanations. One possibility could be that the mutation was not detected because of the low numbers of MC. In this regard, it is noteworthy that the *KIT* mutation D816V is often detectable in such patients only when analyzing highly enriched (sorted or micromanipulated) MC [35–37]. Another possibility could be that this patient exhibited other *KIT* mutations that were not detected by the technique applied. In fact, it has been described that a smaller subgroup of patients with SM display unusual *KIT* mutations [38–40]. Another alternative may be that MC were monoclonal in nature but did not (yet) exhibit a *KIT* mutation.

A number of previous and more recent data suggest that basal serum tryptase levels correlate with the grade of infiltration of the bone marrow by neoplastic MC [41, 42]. Corresponding results were obtained in the present study. In particular, the 2 patients with diagnostic MC infiltrates in their bone marrow had higher serum tryptase levels compared to the other patients in whom no MC infiltrates were detected. However, as mentioned above, a normal serum tryptase level does not exclude the diagnosis SM. Vice versa, an elevated basal serum tryptase does not predict the diagnosis SM with certainty, as also seen in 1 of our patients. In this regard, it should also be mentioned that serum tryptase levels can transiently increase during a severe anaphylactic reaction [16, 17].

Since several patients with SM but also those with MMAS may be at risk to develop life-threatening anaphylaxis (hypotension) under certain conditions like bee or wasp stings [24–26, 32], the clinical management of these patients is of great importance. These patients are usually advised to avoid all agents and situations that may cause anaphylaxis. In addition, it seems reasonable to treat these patients with prophylactic anti-histamines and to prescribe epinephrine self-injectors to be used on demand. In a few patients with SM or MMAS, laboratory features may indicate the presence of an underlying IgE-dependent allergy (although the incidence of allergies may not be higher than that in the general population). In these patients, immunotherapy should be considered. On the other hand, it should also be stated that not all patients with SM develop anaphylaxis after bee or wasp stings.

In summary, our data show that patients with unexplained severe hypotension without specific IgE may suffer from SM or a related condition characterized by only two minor SM criteria. Based on the similarities to SM and the indirect signs of MC clonality, we propose to term the latter condition MMAS. Management and therapy of MMAS should be the same as management and therapy of severe hypotension in patients with SM.

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