Clonal mast cell disorders in patients with systemic reactions to Hymenoptera stings and increased serum tryptase levels

Patrizia Bonadonna, MD,[a,i] Omar Perbellini, MD,[b,i] Giovanni Passalacqua, MD,[c] Beatrice Caruso, MD,[d] Sabrina Colarossi, BS,[e,i] Daniela Dal Fior, MD,[f] Luca Castellani, MD,[g] Chiara Bonetto, MSc,[h] Francesco Frattini, MD,[b] Annarita Dama, MD,[a] Giovanni Martinelli, MD,[e,i] Marco Chilosi, MD,[f] Gianenrico Senna, MD,[a] Giovanni Pizzolo, MD,[b] and Roberta Zanotti, MD[b,i] *Verona, Genoa, Bologna, and Rovereto, Italy*

Background: Anaphylaxis after Hymenoptera stings has been reported in subjects with mastocytosis, but few data exist regarding disease prevalence in populations allergic to these insects.
Objective: The incidence of clonal mast cell (MC) disorders in subjects with both systemic reactions to Hymenoptera stings and increased serum baseline tryptase (sBT) levels was assessed by using bone marrow (BM) aspirates and biopsy specimens.
Methods: Subjects with a history of a systemic reaction caused by a Hymenoptera sting underwent the standard diagnostic work-up for Hymenoptera allergy, and sBT levels were measured. Subjects with an increased sBT level had BM evaluation that included histology/cytology, flow cytometry, and detection of *KIT* mutations.
Results: Forty-four (11.6%) of 379 subjects with systemic reactions had increased sBT levels (>11.4 ng/mL), and 31 (70.5%) of these had a history of anaphylaxis. Thirty-four subjects with increased sBT levels underwent a BM analysis. Histology detected diagnostic or subdiagnostic MC infiltrates in 22 (65%) of 34 patients. Abnormal MCs were identified by means of flow cytometry and cytology in 26 (78.8%) of 33 and 20 (58.8%) of 34 subjects, respectively. A *KIT* mutation was detected in 17 (54.8%) of 31 subjects. The diagnosis was indolent systemic mastocytosis in 21 (61.7%) of 34 subjects and monoclonal MC activation syndrome in 9 (26.5%) of 34 subjects. All subjects with anaphylaxis had one of those 2 disorders.
Conclusion: The concomitant presence of systemic reactions (especially anaphylaxis) after Hymenoptera stings and increased sBT levels strongly suggests that a BM examination is indicated for the diagnosis of clonal MC disease. (J Allergy Clin Immunol 2009;123:680-6.)

Key words: *Hymenoptera venom allergy, anaphylaxis, serum tryptase, mastocytosis, bone marrow*

Mastocytosis is a heterogeneous disorder characterized by the proliferation and accumulation of mast cells (MCs) in the skin, bone marrow (BM), and other tissues. Some of the progenitors are diverted into an MC lineage by the constitutive activation of somatically mutated *KIT* receptor, and this keeps such MCs alive independently of stem cell factor, the ligand for *KIT*.[1] According to the World Health Organization classification, mastocytosis can be classified as cutaneous mastocytosis when limited to the skin and systemic mastocytosis (SM) when MCs infiltrate 1 or multiple extracutaneous organs (mainly the BM).[2] The diagnosis of SM requires the presence of the major criterion (presence of multifocal dense MC infiltrates in BM or other extracutaneous organs) plus 1 minor criterion or 3 minor criteria. Minor criteria include (1) abnormal morphology of extracutaneous MCs (spindle-shaped cells); (2) increased serum tryptase level (>20 ng/mL); (3) expression of CD2, CD25, or both on BM MCs; and (4) detection of a *KIT* mutation at codon 816 in extracutaneous organs.[3,4] SM can also be subdivided into 4 clinical variants: (1) indolent SM (ISM); (2) SM with an associated clonal, hematologic, non-MC lineage disease; (3) aggressive SM; and (4) MC leukemia.[2] The term *monoclonal mast cell activation syndrome* (MMAS) has been proposed for subjects with unexplained recurrent anaphylaxis without skin lesions who only meet 1 (excluding raised tryptase level) or 2 of the minor criteria for SM.[5-7]

Subjects with mastocytosis often experience symptoms after massive MC activation and the release of mediators. Some, but not all, systemic symptoms include hypotension and shock, flushing, headache, generalized pruritus, abdominal pain, diarrhea, or bone and soft tissue pain.[8,9] In SM MCs can be activated by various nonspecific and immunologic stimuli. These latter include an IgE-mediated reaction, as occurs in Hymenoptera venom allergy (HVA). Subjects with HVA and SM have an increased risk

From [a]the Allergy Unit, Verona General Hospital; [b]the Department of Clinical and Experimental Medicine, Haematology Section, University of Verona; [c]Allergy and Respiratory Diseases, DIMI, University of Genoa; [d]Clinical Chemistry and Haematology, Verona General Hospital; [e]the Department of Haematology/Oncology, L. and A. Seragnoli, University of Bologna; [f]the Department of Pathology, University of Verona; [g]the Department of Dermatology, Santa Maria del Carmine Hospital, Rovereto; [h]the Department of Medicine and Public Health, Section of Psychiatry and Clinical Psychology, University of Verona; and [i]Rete Italiana Mastocitosi (RIMA).

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Reprint requests: Giovanni Passalacqua, MD, Allergy and Respiratory Diseases, Department of Internal Medicine, Padiglione Maragliano, L.go R. Benzi 10, 16132 Genoa, Italy. E-mail: passalacqua@unige.it.
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Abbreviations used
BM: Bone marrow
HVA: Hymenoptera venom allergy
ISM: Indolent systemic mastocytosis
MC: Mast cell
MMAS: Monoclonal mast cell activation syndrome
sBT: Serum basal tryptase
SM: Systemic mastocytosis

of severe systemic reactions compared with subjects without SM, as described in several case reports or small series of subjects with HVA.[10-15]

Tryptase is an MC-specific enzyme secreted continuously by these cells as protryptase.[16] Serum baseline tryptase (sBT) correlates with the total MC number. Thus tryptase levels are continuously increased in many subjects with mastocytosis, frequently exceeding 20 ng/mL.[17,18] Routine measurement of sBT levels in subjects with anaphylaxis and suspected HVA has been suggested as a potential screening criterion for SM.[19] Therefore the presence of a BM clonal MC disease was assessed in subjects with systemic reactions caused by HVA and increased serum tryptase levels by obtaining a BM biopsy on which flow cytometric analysis and *KIT* mutation analysis were performed.

METHODS

Subjects

Subjects referred between January 2004 and January 2007 with a history of a systemic reaction caused by a Hymenoptera sting underwent a detailed clinical history, the standard diagnostic work-up for HVA (skin prick tests, intradermal tests, and CAP-RAST assay),[20] and sBT measurement. The severity of the systemic reaction was graded I to IV, according to Mueller's classification.[21] Anaphylaxis, which is included in grade IV reactions, was defined according to the European Academy of Allergology and Clinical Immunology and World Allergy Organization criteria.[22] Serum tryptase levels were measured at least 2 weeks after the sting. Values of greater than 11.4 ng/mL (95th percentile of normal subjects)[19] were considered abnormal. Adult subjects with increased sBT levels consented and had a complete blood cell count, routine biochemistry, abdominal ultrasonography, BM biopsy and aspiration, flow cytometric analysis of BM cells, and determination of the of *KIT* mutation in BM mononuclear cells. A skin biopsy specimen was obtained when urticaria pigmentosa was suspected.

Skin test and serum assays

Skin prick and intradermal tests to *Apis mellifera*, *Vespa crabro*, *Polistes dominulus*, *Vespula* species, and *Bombus terrestris* were performed at least 2 weeks after the sting, as per the recommendations of the European Academy of Allergology and Clinical Immunology.[20,23] Specific IgE levels to the same species were measured by means of CAP-RAST (ImmunoCap 250; Phadia, Uppsala, Sweden). Tryptase levels were determined by using a commercial immunofluorimetric assay (UniCap 100, Phadia).

Histology and immunohistochemistry of BM

BM biopsy specimens were fixed in formalin for 24 hours and embedded in paraffin. Mild decalcification was performed on BM biopsy specimens. Three-micrometer-thick sections were stained with hematoxylin and eosin and Giemsa stains. Immunohistochemistry was performed with antibodies against CD117 (dilution 1:30; rabbit; DAKO, Glostrup, Denmark), CD25 (dilution 1:100; 4C9; Novocastra, Newcastle Upon Tyne, United Kingdom), and MC tryptase (dilution 1:1000; Serotec, Oxford, United Kingdom). Antigen retrieval was conducted in basic buffer (pH 8) at 95°C for 15 minutes (CD2 and CD25) and in acid buffer (pH 6) for 15 minutes in the case of CD117. Pretreatment in a 0.1% trypsin solution was necessary for immunohistochemistry detection of MC tryptase. Reactions were revealed by using the "bond polymer refine" detection system. Immunochemical staining results for MCs were graded as 0 (absence of MCs), 1 (isolated MCs), 2 (small deposits of MCs), or 3 (multifocal dense infiltrates of MCs with more than 15 MCs in aggregates; Fig 1).

BM smears

BM smears stained with May-Grunwald-Giemsa stain were examined for the presence and percentage of atypical MCs according to established criteria.[24]

Multiparametric flow cytometric analysis of BM MCs

Five-color flow cytometry (fluorescein isothiocyanate/phycoerythrin/peridinin-chlorophyll-protein complex/phycoerythrin-cyanine 7/allophycocyanin) was used to identify the presence of pathologic MCs in BM samples. Briefly, after collection, heparinized BM samples were disaggregated by means of passage through a 25-gauge needle. BM cells, about 10^6 cells in 100 μL, were then incubated for 15 minutes at room temperature with a combination of mAbs: CD25/CD2/CD45/CD34/CD117 (BD Biosciences, Milan, Italy). Isotype-matched immunoglobulins were used as a negative control. After red blood cell lysis, samples (at least 10^6 cells) were analyzed with a FACSCanto cytometer (BD Biosciences). For hypocellular samples, 2 or more BM aliquots were stained with the same mAb combination. Analyses were performed with FlowJo software (Tree Star, Ashland, Ore). CD117, CD45, and CD34 were used for gating purposes. Thus the expression of CD25 and CD2 on $CD117^+/CD45^+/CD34^-$ cells was evaluated, as demonstrated in Fig 2.

Analysis of *KIT* mutations

Activating *KIT* mutations[25] were looked for in BM samples. The BM aspirate mononuclear cell fraction containing MCs was separated by using Histopaque (density = 1.077; Sigma, St Louis, Mo) gradient centrifugation and resuspended in RLT solution (Qiagen, Valencia, Calif). RNA was extracted with the RNeasy RNA Isolation Mini Kit (Qiagen). For *KIT* mutation analyses, an RT-PCR product of 287 bp spanning codons 763 to 858 (exons 16-18) and corresponding to the catalytic domain and activation loop in the enzymatic site was digested with the restriction enzyme *Hin*fI and assessed by using an RFLP assay according to the method of Akin.[26] Fragments with predicted sizes were visualized after separation on a 4% agarose gel and ethidium bromide staining. Digestion of the wild-type RT-PCR product with this enzyme yields fragments of 171 and 116 bp. An A-to-T substitution (GAC>GTC) at *KIT* nucleotide 2468 (within the 171-bp fragment), resulting in the D816V mutation, introduced a new *Hin*fI recognition site and production of restriction digest fragment sizes of 157 and 14 bp.

For each sample scored as wild-type by means of RFLP analysis, the RT-PCR product of 287 bp (exons 16-18) was screened by means of denaturing HPLC combined with direct sequencing to detect enzymatic site mutations other than D816V.[26] Patient samples that did not harbor ES-type mutations were further investigated by means of denaturing HPLC analysis of an RT-PCR product of 350 bp spanning codons 510 to 626 (exons 10, 11, and 12) and corresponding to the transmembrane domain and to the juxtamembrane domain to detect RT mutations.

Statistical analysis

The Mann-Whitney *U* test was used to compare sBT levels according to sex and age. The same comparisons according to allergy tests and grades

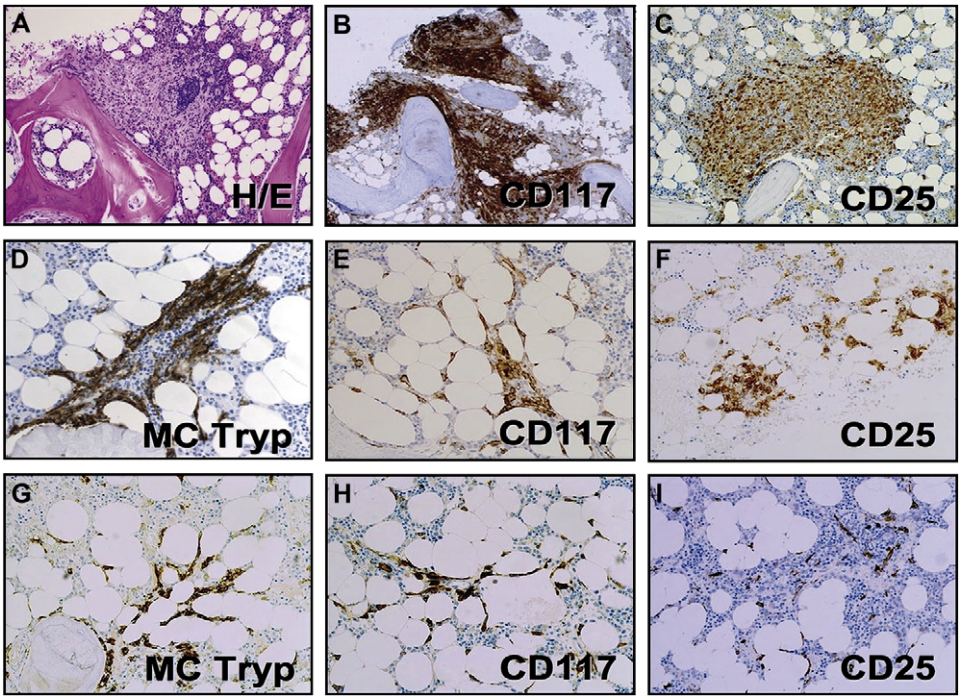

FIG 1. BM histology and immunohistochemistry. BM biopsy specimens were stained with hematoxylin and eosin (*H/E*; **A**) and with mAbs against CD117 (**B**, **E**, and **H**), MC tryptase (*Tryp*; **D** and **G**), and CD25 (**C**, **F**, and **I**). Immunohistochemistry staining for MCs allowed a quantitative evaluation and a classification as follows: score 3, multifocal dense infiltrates of MCs with greater than 15 MCs in aggregates (Fig 1, *A-C*); score 2, small deposits of MCs (Fig 1, *D-F*); and score 1, isolated MCs (Fig 1, *G-I*).

of allergic reaction were assessed by using the Kruskall-Wallis test. The χ^2 test was used to compare subjects according to sex, age, allergy test results, and grade of allergic reaction with respect to normal and increased sBT levels. All tests were 2-tailed, with a P value of less than .05 considered significant. The analyses were performed with SPSS 14.0 for Windows (SPSS, Inc, Chicago, Ill).

RESULTS

Clinical and laboratory data

Three hundred seventy-nine subjects (266 male subjects; age range, 6-79 years; median age, 43 years) with a history of a systemic reaction to a Hymenoptera sting were evaluated. The demographic and clinical data are summarized in Table I. Four of 379 subjects had negative skin and CAP test results to Hymenoptera. These 4 subjects had significantly higher sBT levels than subjects with HVA (29.1 ± 8.8 vs 6.7 ± 8.9 ng/mL, $P = .001$). Overall, the concentration of sBT positively correlated with the severity of sting reactions ($P = .01$, Kruskall-Wallis test, data not shown).

Forty-four (11.6%) of 369 subjects had sBT levels of greater than 11.4 ng/mL, only 1 of whom was less than 18 years of age. The comparative analysis according to the sBT level is shown in Table I. Thirty-one (70.5%) of 44 subjects with increased sBT levels had a history of anaphylaxis versus 93 (27.7%) of 335 of those with normal sBT levels ($P < .0001$). Two subjects had a previous diagnosis of urticaria pigmentosa, which was confirmed by means of skin histology. Skin biopsies were performed in 5 other subjects with suspected urticaria pigmentosa. The diagnosis was confirmed in 2 of these subjects, and in 1 subject a subdiagnostic infiltrate of MCs was detected. Thirty-four of 43 adult subjects with increased sBT levels consented to BM aspiration and biopsy.

BM biopsy

BM biopsy was performed in 33 of 34 subjects because 1 had vasovagal syncope after aspiration and could not conclude the procedure. BM biopsy demonstrated the typical dense multifocal MC infiltrates (score = 3) positive for tryptase, CD117, and CD25 in 14 of 33 subjects. All subjects with positive results had at least 1 previous episode of anaphylaxis. In 8 other subjects a subdiagnostic MC infiltrate was detected, which was characterized by atypical isolated or small aggregates of $CD117^+$ and $CD25^+$ MCs (score = 1-2). The BM biopsy result was negative in 11 subjects. Examples of stained BM biopsy specimens are shown in Fig 1.

Morphology of MCs in BM smears

BM smears showed more than 25% of MCs with spindle-shaped morphology, which is consistent with atypical MC type I, in 20 (58.8%) of 34 subjects. In the remaining 13 subjects MCs were normal. The BM smear from 1 patient displayed a severe hypocellularity.

Flow cytometric analysis of MCs

This analysis was carried out in 33 subjects because in 1 subject it was not available at the time of BM aspiration. In 26 (78.8%) of 33 tested subjects, BM MCs stained positive for CD25 and CD2. The median percentage of $CD117^{++}/CD34^-/CD25^+$ cells from

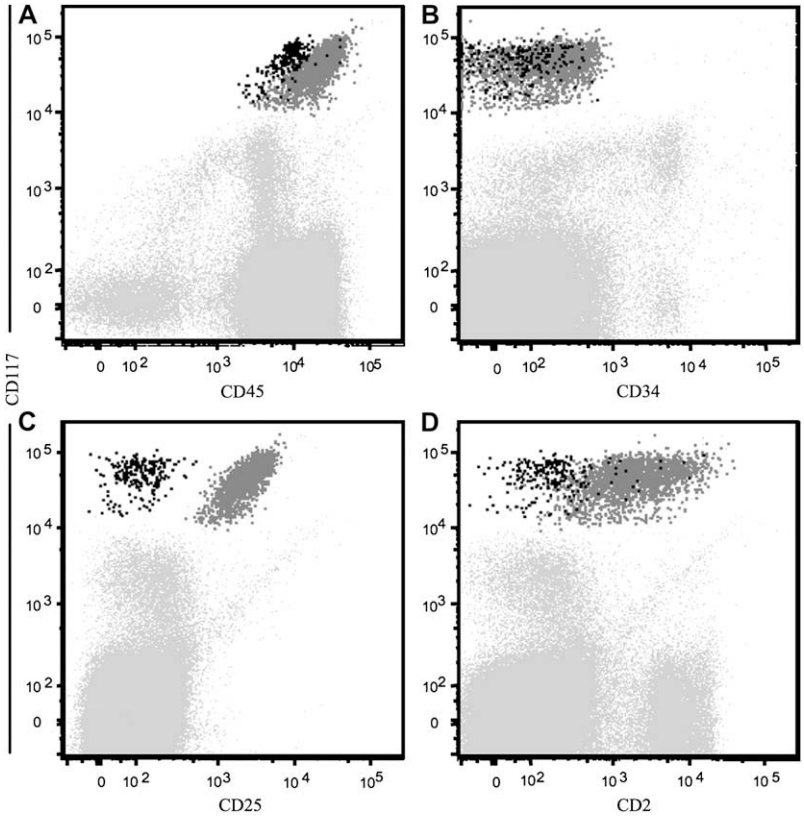

FIG 2. Flow cytometric analysis of BM MCs. MCs were identified as $CD117^{++}/CD45^{+}/CD34^{-}$ cells (**A** and **B**, *dot plots*). CD25 and CD2 antigens were clearly aberrantly expressed on neoplastic MCs *(dark gray)* but not on normal MCs (*light gray*; **C** and **D**, *dot plots*).

the total BM $CD45^{+}$ cells was 0.049% (range, 0.004% to 1.38%). One sample could not be evaluated because of severe hypocellularity.

KIT mutation analysis

KIT mutation analysis was performed in 31 of 34 subjects but not in 3 subjects because of the unavailability of the test at the time of BM aspiration. Seventeen (54.8%) of 31 tested subjects displayed the D816V *KIT* point mutation. Of the 13 available D816V-negative samples, denaturing HPLC analyses showed an abnormal elution profile in 4 subjects. The activating *KIT* mutation D816H was detected in 1 subject, and in 3 subjects direct sequencing confirmed the presence of a M541L polymorphism, resulting in a Met-to-Leu amino acid substitution at codon 541 in the transmembrane domain.

Final diagnosis

A final diagnosis of ISM was confirmed in 21 (61.8%) of 34 subjects based on the above-mentioned results (Table II). Fourteen subjects fulfilled both major and minor criteria, and 7 subjects satisfied 3 minor criteria. HVA was the presenting symptom of disease in 19 (90.5%) of 21 subjects, and in 4 subjects urticaria pigmentosa was documented. Eighteen of the 21 subjects had anaphylaxis caused by a Hymenoptera sting. Median sBT levels were higher in subjects with skin involvement (79.6 ng/mL; range, 27.1-103.0 ng/mL) than in subjects only with BM involvement (21.2 ng/mL; range, 12.6-42.0 ng/mL; $P = .07$).

Less than 3 minor criteria (2 criteria in 7 subjects and 1 criterion in 2 subjects) were fulfilled in 9 (26.5%) of 34 subjects. MCs coexpressing CD25/CD2, a *KIT* mutation at codon 816, or both were documented in all of these subjects. Thus these 9 subjects were classified as having MMAS. In 2 of 9 subjects, incomplete tests were performed. Six (66.6%) of 9 subjects had a history of anaphylaxis caused by Hymenoptera venom. The median sBT level was 17.6 ng/mL (range, 12.7-23.8 ng/mL).

Among the 4 subjects without HVA (negative allergy test result), 3 had ISM (n = 3), and 1 had MMAS. The remaining 4 (11.8%) of 34 subjects had negative results for clonal MC disorders. Their median sBT level was 16.3 ng/mL (range, 14.4-20.1 ng/mL), and none had a history of anaphylaxis.

DISCUSSION

Systemic reactions to Hymenoptera stings with variable severity occur in up to 5% of the adult population in Europe and the United States. Subjects with mastocytosis might experience more severe reactions after Hymenoptera stings,[11-13,16-18,27,28] and HVA was the most common trigger of anaphylaxis in a large cohort of subjects with SM.[28] An increased sBT level is associated with a history of more severe sting reactions,[12,14,29] but persistently increased sBT levels are also a marker of increased number or activity of MCs, as in mastocytosis.[30] In 2 large case series, HVA was reported in 5% and 19% of the subjects with

TABLE I. Demographic, clinical, and laboratory data of the 379 patients with systemic reactions caused by Hymenoptera stings grouped according to normal or increased basal sBT levels

	Patients with normal sBT levels, no. (%)	Patients with increased sBT levels (>11.4 ng/mL), no. (%)	*P* value
Total	335 (88.4)	44 (11.6)	—
Sex			
Male	233 (69.5)	33 (75.0)	.290*
Female	102 (30.5)	11 (25.0)	—
Ratio	2.28	3.00	
Age (y)			
Mean (SD)	42.3 (16.3)	48.1 (15.6)	.038† §
Median (range)	42.5 (6-78)	48.1 (17-77)	
Pediatric (<18 y)	29 (8.7)	1 (2.3)	.230‡
Adult	306 (91.3)	43 (97.7)	
Allergy tests for HVA			
Negative	0 (0.0)	4 (9.1)	.0001‡
Positive to:	335 (100)	40 (90.9)	
Apis mellifera	68 (20.3)	7 (15.9)	
Vespula species	199 (59.4)	23 (52.3)	.743‡
Polistes dominulus	67 (20.0)	10 (22.7)	
Vespa crabro	1 (0.3)	0 (0.0)	
Grade of allergic reaction			
I	33 (9.8)	2 (4.5)	.0001‡
II	93 (27.7)	7 (15.9)	
III	116 (34.6)	4 (9.1)	
IV	93 (27.7)	31 (70.5)	

*χ^2 Test.
†Mann-Whitney test.
‡Fisher exact test.
§$P = .130$ by excluding pediatric patients.

mastocytosis,[27,28] and the frequency of mastocytosis in subjects with HVA was reported to range from 1% to 2.6%.[19,31] Therefore sBT level can be the indirect link between SM and HVA. Nevertheless, routine screening of sBT levels in subjects with systemic reactions caused by Hymenoptera stings is not a common medical practice. The diagnostic work-up for SM in such subjects is usually limited to those with typical skin lesions because cutaneous mastocytosis or SM with skin involvement is believed to represent the majority of the subjects with HVA,[11,12,18,28] although de Olano et al[27] reported that 76% of subjects with HVA and SM do not have skin involvement.

An abnormally increased sBT level was identified in 44 (11.6%) of 379 subjects in this study who had a history of a systemic reaction to a Hymenoptera sting, which is in agreement with previous reports.[13,15,19,32] Moreover, sBT levels positively correlated with the severity of the systemic reaction because 31 (70.5%) of 44 subjects with increased sBT levels had a previous history of anaphylaxis.[13,15,33] An MC clonal disorder was diagnosed in 30 (8%) of 379 of the subjects with systemic reactions to Hymenoptera stings. This could underestimate the real prevalence because the subjects with normal sBT levels did not undergo BM analysis. In particular, SM was diagnosed in 22 (5.8%) of 379 subjects, a value higher than in previous reports.[19,31] This could be explained by the diagnostic protocol used and the cutoff value for sBT of 11.4 ng/L[19,32,33] instead of 20 ng/L, as recommended in guidelines.[34] In our subjects only 50% of subjects with ISM/MMAS would have received diagnoses with this value instead of 88% with the value of 11.4 ng/L. This cutoff provided a false-positive result in 4 of 34 subjects, none of whom had anaphylaxis. On the other hand, no conclusion on the overall sensitivity or specificity of sBT can be derived because patients with normal sBT levels were not studied for clonal MC disorders. In subjects with a history of anaphylaxis, the diagnosis of clonal MC disorders was made in all cases.

This is the first study with a flow cytometric evaluation of BM MCs and *KIT* mutation analysis in subjects with HVA and increased sBT levels. BM immunochemistry revealed a diagnostic or subdiagnostic MC infiltrate in 65.9% of subjects, morphologic evaluation of BM smears documented greater than 25% spindle-shaped MCs in 58.8% of subjects, cytometry demonstrates an aberrant BM MC phenotype in 78.8% of subjects, and activating *KIT* mutations were documented in 58.1% of subjects. Identifying the *KIT* mutations increases the diagnostic value of BM study. In particular, 6 (20%) of 30 cases of SM/MMAS could not have been diagnosed without flow cytometry, *KIT* mutation analysis, or both.

Subjects with anaphylaxis after wasp or bee stings and negative allergy test results might have unrecognized mastocytosis.[35] In these subjects investigations for major and minor SM criteria are recommended, regardless of sBT level or the presence of skin lesions.[5] In our series all subjects without IgE-mediated HVA had increased sBT levels and a diagnosis of a clonal MC disorder. These results confirm the existence of a link between mastocytosis and systemic reactions to Hymenoptera stings, even in subjects with negative allergy test results. Moreover, BM clonal MC disorder was documented in the great majority of subjects with only increased sBT levels and HVA, especially if they had anaphylaxis. The main limitation of this study is that the presence of an occult MC clonal disease cannot be excluded in those subjects with HVA and previous anaphylaxis but tryptase levels of less than 11.4 ng/mL. Such subjects comprised 24.5% of our patients. Although it would be relevant to know the prevalence of the disease in all subjects with anaphylaxis, the diagnosis would require an invasive procedure (ie, BM aspirate and biopsy) with no real indication, and this might be impractical. Assessment of sBT levels is an inexpensive, reliable, and simple screening test for mastocytosis in subjects with a positive history of a systemic reaction to Hymenoptera stings. Results indicate that an sBT level greater than normal is highly suggestive of a clonal MC disorder. In particular, anaphylaxis after Hymenoptera stings associated with a persistent increase in sBT level can be considered a strong indicator of clonal MC proliferation and thus would deserve BM histology/cytology, multiparametric cytometry, and *KIT* mutation assessment to detect an occult BM involvement. A careful cost/benefit analysis is necessary but would be relevant for those subjects with mild systemic reactions who are currently not candidates for venom immunotherapy. Moreover, an early diagnosis of occult SM would help to prevent further severe reactions and, possibly, other complications of SM, including osteoporosis or bone lesions.

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TABLE II. Clinical characteristics, BM findings, and final diagnosis of 34 patients with systemic reactions caused by Hymenoptera stings and increased sBT levels

Patient no.	Sex	Age (y)	Allergy test (specificity)	Allergic reaction (grade)	BM biopsy (score = 3)	BM MC $CD25^+$ (%)*	Activating *KIT* mutation (BM)	Spindle-shaped BM MCs (>25%)	sBT (ng/mL)	Skin biopsy	MC disorder diagnosis
1	F	31	*Vespula* species	I	Negative	Negative	Negative‡	Negative	14.4	—	—
2	M	46	Negative	I	Negative	**0.008**	Negative	Negative	**27.0**	—	MMAS
3	M	41	*Vespula* species	II	Negative	Negative	Negative	Negative	16.3	—	—
4	M	35	*Apis* species	II	Negative	Negative	Negative	Negative	**20.1**	—	—
5	M	62	*Vespula* species	II	Negative	Negative	**D816V**	Negative	**23.3**	—	MMAS
6	M	51	*Vespula* species	II	Negative	Negative	**D816H**	Negative	17.4	—	MMAS
7	M	69	*Apis* species	III	Negative	Negative	Negative	Negative	12.7	—	—
8	M	62	*Vespula* species	III	Negative‡	**0.027**	**D816V**	**Positive**	**31.6**	—	ISM
9	F	63	Negative	III	**Positive**	**0.009**	Negative	**Positive**	**22.7**	—	ISM
10	M	19	*Vespula* species	III	Negative	**0.004**	**D816V**	Negative	**20.1**	—	ISM
11	M	55	*Vespula* species	IV	Negative‡	**0.047**	Negative	Negative	12.7	—	MMAS
12	M	69	*Vespula* species	IV	Negative	**0.04**	NE	**Positive**	16.6	—	MMAS
13	M	51	*Vespula* species	IV	Negative	**0.008**	Negative	Negative	**23.8**	—	MMAS
14	M	69	*Vespula* species	IV	Negative	NE	**D816V**	Negative	18.4	—	MMAS
15	M	41	*Vespula* species	IV	NT	**0.03**	Negative†	**Positive**	13.2	—	MMAS
16	M	32	*Polistes dominulus*	IV	Negative‡	**0.017**	Negative	**Positive**	17.6	—	MMAS
17	F	58	Negative	IV	**Positive**	**0.035**	**D816V**	**Positive**	**42.0**	Negative	ISM
18	M	45	*Polistes dominulus*	IV	**Positive**	**0.28**	Negative†	**Positive**	12.6	Negative	ISM
19	M	74	*Polistes dominulus*	IV	**Positive**	**0.079**	Negative	Negative	**21.2**	—	ISM
20	M	64	*Polistes dominulus*	IV	Negative‡	**0.12**	**D816V**	**Positive**	**24.2**	—	ISM
21	M	50	*Polistes dominulus*	IV	Negative‡	**0.008**	**D816V**	**Positive**	17.6	—	ISM
22	F	39	*Vespula* species	IV	Negative‡	**0.059**	**D816V**	**Positive**	18.0	—	ISM
23	M	37	*Vespula* species	IV	**Positive**	**0.15**	**D816V**	**Positive**	**20.1**	—	ISM
24	M	48	*Vespula* species	IV	**Positive**	**0.03**	NT	NE	16.5	—	ISM
25	F	52	*Polistes dominulus*	IV	**Positive**	**0.24**	**D816V**	**Positive**	13.4	—	ISM
26	F	76	*Vespula* species	IV	**Positive**	NT	NT	Positive	**30.0**	—	ISM
27	F	68	*Polistes dominulus*	IV	Negative‡	**0.02**	**D816V**	Negative	**28.6**	—	ISM
28	M	28	Vespula species	IV	**Positive**	**0.22**	**D816V**	**Positive**	**26.4**	—	ISM
29	M	39	*Apis* species	IV	**Positive**	**0.335**	**D816V**	**Positive**	**103**	Positive	ISM
30	M	33	*Polistes dominulus*	IV	**Positive**	**0.2**	**D816V**	**Positive**	**56.2**	Positive	ISM
31	M	43	*Vespula* species	IV	**Positive**	**0.8**	**D816V**	**Positive**	**103**	Positive	ISM
32	M	43	*Polistes dominulus*	IV	**Positive**	**1.38**	**D816V**	**Positive**	**27.1**	Positive	ISM
33	F	58	Negative	IV	Negative‡	**0.05**	**D816V**	**Positive**	**24.8**	Negative‡	ISM
34	F	48	*Vespula* species	IV	**Positive**	**0.20**	NT	**Positive**	13.8	—	ISM

Items in boldface indicate criteria fulfilled for diagnosis of SM.
NE, Not evaluated; *NT*, not tested.
*Evaluated by means of flow cytometry.
†M541L *KIT* polymorphism.
‡Subdiagnostic MC infiltrate.

Clinical implications: Subjects with anaphylaxis caused by Hymenoptera stings independent of IgE sensitization and increased serum tryptase levels should be carefully assessed for MC clonal disorders.

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