Clonal mast cell disorders in patients with severe Hymenoptera venom allergy and normal serum tryptase levels

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Background: Systemic mastocytosis is a clonal mast cell (MC) disease that can lead to potentially fatal anaphylactic reactions caused by excessive MC mediator release. The prevalence of mastocytosis in patients with Hymenoptera venom allergy is high, and thus the disease should be suspected in patients with severe reactions caused by Hymenoptera stings and increased serum basal tryptase (SBT) levels.

Objective: We sought to evaluate the presence of clonal MC disorders in patients seen at our mastocytosis center with Hymenoptera sting–induced anaphylaxis, documented hypotension, absence of urticaria pigmentosa, and normal SBT levels.

Methods: Twenty-two patients with Hymenoptera sting–induced anaphylaxis, without skin lesions, and with tryptase levels of less than 11.4 ng/mL underwent bone marrow evaluation. Bone mineral density was assessed in those patients with ascertained mastocytosis.

Results: In 16 of 22 patients, a diagnosis of indolent mastocytosis could be established, and 1 patient had a monoclonal MC activation syndrome. Patients with mastocytosis had higher SBT levels ($P = .03$) but only rarely had angioedema/urticaria associated with hypotension ($P = .004$).

Conclusions: The absence of urticaria or angioedema in severe reactions to Hymenoptera stings with hypotension might represent the most relevant factor in identifying patients with mastocytosis, regardless of their serum tryptase levels.

(J Allergy Clin Immunol 2015;■■■:■■■-■■■.)

Key words: *Systemic mastocytosis, anaphylaxis, tryptase, Hymenoptera venom allergy*

Systemic mastocytosis (SM) is a heterogeneous hematologic disease characterized by the proliferation and accumulation of mast cells (MCs) in different tissues, with a preferential localization in bone marrow (BM) and skin.[1] The diagnosis of SM requires the presence of the major criterion (ie, multifocal dense MC infiltrates in BM or other extracutaneous organs) plus 1 minor criterion or 3 minor criteria. Minor criteria include abnormal morphology of extracutaneous MCs (spindle-shaped cells); an increased serum basal tryptase (SBT) level of greater than 20 ng/mL; expression of CD2, CD25, or both on BM MCs; and detection of a *KIT* mutation at codon 816 in extracutaneous organs. SM is subdivided into 4 clinical variants: indolent systemic mastocytosis (ISM), SM with an associated clonal non-MC lineage disease, aggressive SM, and MC leukemia.[2]

Patients with SM can experience symptoms because of a massive MC activation and release of mediators (eg, generalized pruritus, urticaria/angioedema, abdominal pain, and anaphylaxis).[2] It has been noticed that there is a preferential association between Hymenoptera venom allergy (HVA) and SM because the prevalence of SM in patients with HVA is relatively high.[3,4] In addition, symptoms of HVA are a common clinical presentation in patients with ISM without skin involvement.[4-6] An increased SBT appears to be a criterion useful to identify patients eligible for BM evaluation, who are suspected of having SM.[7] In a previous study 88.2% of patients with systemic reactions to Hymenoptera stings and SBT levels of greater than 11.4 ng/mL were given a diagnosis of a clonal MC disorder.[4,8] On this basis, some authors suggested that the current cutoff level for SBT of greater than 20 ng/mL should be decreased, at least in patients with a history of systemic reaction to Hymenoptera stings. On the other hand, a clonal mast cell disorder (CMD) cannot be excluded in patients with systemic severe HVA but normal SBT levels.[9]

Only a few cases of CMD without specific skin lesions and normal SBT levels have been reported in patients with HVA.[10] Such patients represent a challenge for specialists when a BM biopsy must be considered. To better elucidate this point, we evaluated the presence of CMD in patients with a severe systemic reaction to Hymenoptera venom, a normal SBT level, and the absence of urticaria pigmentosa using a BM biopsy.

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Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

Received for publication May 31, 2014; revised November 11, 2014; accepted for publication November 19, 2014.

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0091-6749/$36.00

http://dx.doi.org/10.1016/j.jaci.2014.11.035

Abbreviations used
BM: Bone marrow
CMD: Clonal mast cell disorder
HVA: Hymenoptera venom allergy
IQR: Interquartile range
ISM: Indolent systemic mastocytosis
MC: Mast cell
ROC: Receiver operating curve
SBT: Serum basal tryptase
SM: Systemic mastocytosis

METHODS

Patients and diagnostic procedures

Patients referred to the Verona Multidisciplinary Outpatient Clinics for Mastocytosis for suspicion of CMD after a severe reaction to a Hymenoptera sting were included between September 2012 and November 2013. Patients with a history of 1 or more episodes of anaphylaxis with documented hypotension, loss of consciousness,[11] or both without suggestive skin lesions (ie, urticaria pigmentosa) and with SBT levels of less than 11.4 ng/mL were identified. All patients provided informed consent to allergy and hematologic procedures. There was no need for ethics committee approval because all the diagnostic tests are part of the standard procedure when a differential diagnosis of severe anaphylaxis is required.

The diagnosis of HVA was made according to current guidelines with skin prick tests, intradermal tests, and specific IgE assays.[12] Skin tests were performed with a commercial extract of *Polistes dominula*, *Vespa crabro* (Allergo Diagnostics, Florence, Italy), *Vespula* species, or honeybee (Stallergenes, Antony, France). Four venom concentrations (100 μg/mL for skin prick tests and 0.02 mL of 0.01, 0.1, and 1.0 μg/mL for intradermal tests) were used plus a negative (NaCl 0.9%) and a positive (10 mg/mL histamine dihydrochloride) control. Serum specific IgE levels for the same venoms and serum tryptase levels were assayed by using ImmunoCAP (Thermo Fischer Scientific, Uppsala, Sweden) at least 2 weeks after the last acute episode.

All patients underwent BM evaluation, including smear and biopsy, detection of the D816V *KIT* mutation, and flow cytometric analysis.[2,4,13,14] For analysis of MC immunophenotype, we used a highly sensitive, multiparameter flow cytometric approach, as previously reported.[4,13] We stained BM cells with a combination of 5 mAbs, CD45, CD117, CD34, CD25, and CD2, and then acquired at least 10^6 cells per sample using a FACSCanto cytometer (BD Biosciences, San Jose, Calif) and up to 6×10^6 cells in the case of a low MC count.[13,14] After cell acquisition, analysis was performed with DIVA software (BD Biosciences). MCs were identified as $CD45^+/CD117^{++}/CD34^-$ cells. Thus the abnormal expression of CD25, CD2, or both on MCs was evaluated. Irrelevant isotype-matching mAbs were used as negative controls. The percentage of total and abnormal MCs was calculated on $CD45^+$ cells. The D816V *KIT* mutation was detected on total RNA from mononuclear cell fractions of BM samples by using Ficoll/Hypaque density gradient centrifugation. Total RNA was extracted from BM samples by using Trizol Reagent (Invitrogen, Life Technology, Paisley, United Kingdom). cDNA was synthesized from 1 μg of total RNA by using M-MLV Reverse Transcriptase (Invitrogen, Life Technology). D816V *KIT* mutation detection was conducted with allele-specific ARMS–RT-qPCR with primers and instrumentation proposed by Lawley et al.[14] The sensitivity of the method was assessed by using serial 10-fold dilutions (from 10% to 0.0001% dilutions) of RNA from an HMC-1–positive control (kindly provided by the Mayo Foundation for Medical Education and Research) that is heterozygous for the D816V *KIT* mutation. The diagnosis of SM was made according to current World Health Organization guidelines.[1]

Bone mineral density was also measured in all patients with ascertained CMDs by using dual x-ray absorptiometry (QDR Delphi; Hologic, Bedford, Mass) at the lumbar spine (L1-L4) and at the total proximal hip. Osteoporosis was defined as a lumbar spine or hip bone density T score of −2.5 SD or less, according to the traditional World Health Organization criteria.[15] In addition, all patients underwent total spinal and pelvic radiography and abdominal ultrasonography.

Statistical analysis

Statistical analysis was performed with SPSS 17.0 software (SPSS, Chicago, Ill). Continuous variables were expressed mainly as medians ± interquartile ranges (IQRs) because they were not normally distributed. Categorical variables were expressed as percentages. Differences of continuous variables between groups were calculated by using the Mann-Whitney test. Differences of categorical variables between groups were calculated by using the Fisher exact test because of the small sample size. A P value of less than .05 was considered significant. Multivariate analysis was performed by using binary logistic regression. The performance of different variables in predicting disease was defined by the receiver operating curve (ROC).

RESULTS

Twenty-two patients (16 male patients; median age, 61 years; age range, 35-75 years) with at least 1 episode of anaphylaxis with ascertained hypotension after Hymenoptera stings were included. The clinical characteristics of those 22 patients and the results of diagnostic workup are summarized in Tables I and II, respectively. Eleven of 22 patients experienced more than 1 anaphylactic episode (median, 3 episodes; IQR, 2 episodes). Twelve of 22 had HVA to *Vespula* species, 6 to *P dominula*, 2 to *V crabro*, and 2 to honeybee. The diagnosis of ISM was established in 15 of 16 patients plus 1 case of monoclonal MC activation syndrome (clonal BM MCs in the absence of sufficient criteria for SM and typical skin lesions). The median SBT level of the 16 patients given a diagnosis of CMD was 8.6 ng/mL (IQR, 2.27 ng/mL; Tables I and II). A D816V mutation of the *KIT* gene was documented in all but one case (Table II). The median percentage of BM MCs expressing CD25 among BM mononuclear cells was 0.008% (range, 0.001% to 0.21%). Notably, in all cases the diagnosis of ISM was based only on minor criteria because BM biopsy specimens did not show compact MC aggregates. Of the 16 patients with CMD, 4 had evidence of osteoporosis (2 with multiple vertebral fractures) at densitometry and spinal radiography. Only 3 of 16 patients with CMDs reported other mediator-related symptoms (flushing episodes in 2 patients and gastritis in 1 patient).

Those patients with ascertained CMDs have slightly higher SBT levels ($P = .03$) and rarely showed angioedema/urticaria during the anaphylactic episode ($P = .004$). Despite the frequency of multiple anaphylactic episodes being higher in patients with CMDs, statistical significance was not reached, probably because of the small number. The sensitization profile was similar between patients with and without a CMD diagnosis (Table III).

Logistic regression for those variables significantly associated with CMDs in univariate analysis confirmed that absence of angioedema/urticaria was more frequent in patients with a CMD ($P = .023$). SBT showed a trend toward higher levels only in patients with CMDs ($P = .073$, Table IV). ROCs for angioedema/urticaria and tryptase levels were constructed (Fig 1). We obtained significant results for the absence of urticaria/angioedema (area under the curve, 0.854; $P = .012$), which seems to be a good predictor of CMDs (specificity, 83.3%; sensitivity, 87.5%). The ROC for tryptase showed a good performance in predicting the final diagnosis (area under

TABLE I. Clinical characteristics of patients with severe anaphylaxis and normal tryptase levels and without urticaria pigmentosa

Patient code	Sex	Age (y)	SBT (ng/mL)	HVA	No. of systemic reactions to stings	Densitometry lumbar T score	Other mediator-related symptoms	Final diagnosis
29922	F	65	9.0	*Polistes dominula*	4	−1.6	Absent	MMAS
27135	M	61	10.8	*Vespula* species	2	−4.4*	Absent	ISM
28917	M	60	8.4	*Polistes dominula*	4	−2.5*	Absent	ISM
30340	F	64	10	*Polistes d*	2	−2.5	Absent	ISM
31514	M	63	8.9	*Polistes dominula*	1	−0.9	Absent	ISM
31507	M	65	10.7	*Vespula* species	4	0.7	Absent	ISM
31951	M	50	7.8	*Vespula* species	1	−0.3	Absent	ISM
33022	M	51	8.0	*Crabro* species	1	−1.7	Absent	ISM
33139	M	52	7.2	*Polistes dominula*	1	−1.7	Absent	ISM
33195	M	51	6.6	*Vespula* species	1	0.2	Absent	ISM
33337	M	39	9.4	*Polistes dominula*	3	−0.9	Absent	ISM
33445	M	66	7.5	*Vespula* species	3	1.4	Absent	ISM
33562	M	35	4.2	*Vespula* species	3	−0.5	Absent	ISM
33543	F	67	11.2	*Vespula* species	1	1.1	Flushing	ISM
33594	M	42	8.3	*Apis* species	4	−1.8	Flushing	ISM
33792	M	74	8.8	*Vespula* species	2	−0.4*	Gastritis	ISM
27760†	F	55	4.0	*Vespula* species	5	ND	Absent	Non-CMD
32456†	M	69	7.9	*Vespula* species	1	ND	Absent	Non-CMD
32829†	F	54	8.5	*Vespula* species	1	ND	Absent	Non-CMD
33628†	M	61	7.7	*Vespula* species	1	−1.6	Absent	Non-CMD
33655†	M	65	6.3	*Crabro* species	1	ND	Absent	Non-CMD
33924†	F	75	6.5	*Apis* species	1	ND	Absent	Non-CMD

F, Female; *M*, male; *MMAS*, monoclonal mast cell activation syndrome; *ND*, not determined.
*Vertebral fractures
†Patients without an SM diagnosis.

TABLE II. BM study results of 22 patients with severe HVA and normal SBT levels

Patient	Major WHO criteria	*KIT* mutation	MC CD25$^+$ (% of BM MNCs)	MC CD2$^+$ (% of BM MNCs)	>25% Atypical BM MCs	Final diagnosis
29922	Absent	Negative	0.001	0.001	Negative	MMAS
27135	Absent	D816V	0.210	0.210	Positive	ISM
28917	Absent	D816V	0.006	0.006	Positive	ISM
30340	Absent	D816V	0.032	0.032	Positive	ISM
31514	Absent	D816V	0.002	0.002	Positive	ISM
31507	Absent	D816V	0.078	0.078	Positive	ISM
31951	Absent	D816V	0.006	0.006	Positive	ISM
33022	Absent	D816V	0.040	0.030	Positive	ISM
33139	Absent	D816V	0.004	0.004	Positive	ISM
33195	Absent	D816V	0.001	0.0008	Positive	ISM
33337	Absent	D816V	0.030	0.030	Positive	ISM
33445	Absent	D816V	0.009	0.009	Positive	ISM
33562	Absent	D816V	0.002	0.002	Positive	ISM
33543	Absent	D816V	0.004	0.004	Positive	ISM
33594	Absent	D816V	0.023	0.023	Positive	ISM
33792	Absent	D816V	0.130	0.130	Positive	ISM
27760*	Absent	Negative	0.000	0.000	Negative	Non-CMD
32456*	Absent	Negative	0.000	0.000	Negative	Non-CMD
32829*	Absent	Negative	0.000	0.000	Negative	Non-CMD
33628*	Absent	Negative	0.000	0.000	Negative	Non-CMD
33655*	Absent	Negative	0.000	0.000	Negative	Non-CMD
33924*	Absent	Negative	0.000	0.000	Negative	Non-CMD

MNCs, Mononucleated cells; *WHO*, World Health Organization.
*Patients without an SM diagnosis.

TABLE III. Clinical and demographic data of 16 patients with ascertained CMDs compared with those of 6 patients without CMDs

	CMD group (n = 16)	Non-CMD group (n = 6)	*P* value
Male sex, no. (%)	13 (81.2)	3 (50.0)	NS
Age (y), median (IQR)	60.5 (15)	63 (16)	NS
Tryptase (ng/mL), median (IQR)	8.6 (2.27)	7.1 (2.33)	.033
Negative allergy test result, no. (%)	1 (6.2)	1 (16.7)	NS
Angioedema plus urticaria, no. (%)	2 (12.5)	5 (83.3)	.004
Multiple anaphylaxis (>1 per patient)	10 (62.5)	1 (16.7)	NS
REMA score ≥2	14 (87.5)	1 (16.7)	.004
Hymenoptera sensitization			
Vespula species	8 (50)	4 (66.6)	NS
Polistes species	6 (37.5)	0	NS
Vespa crabro	1 (6.25)	1 (16.7)	NS
Apis species	1 (6.25)	1 (16.7)	NS

NS, Not significant.

TABLE IV. Multivariate analysis

	B	SE	Wald	*P* value	Exp (B)
Absence of urticaria/ angioedema	4.991	2.201	5.143	.023	147.15
SBT	1.053	0.587	3.215	.073	2.867

the curve, 0.802; $P = .033$). A cutoff of 7.95 ng/mL showed a sensitivity of 68.8% and a specificity of 83.3%. Because of the small number of patients, it was not possible to design more reliable predictive models.

DISCUSSION

The epidemiologic association between HVA and CMDs is well known[3] and accepted and represents a diagnostic challenge for both allergists and hematologists. In the presence of severe

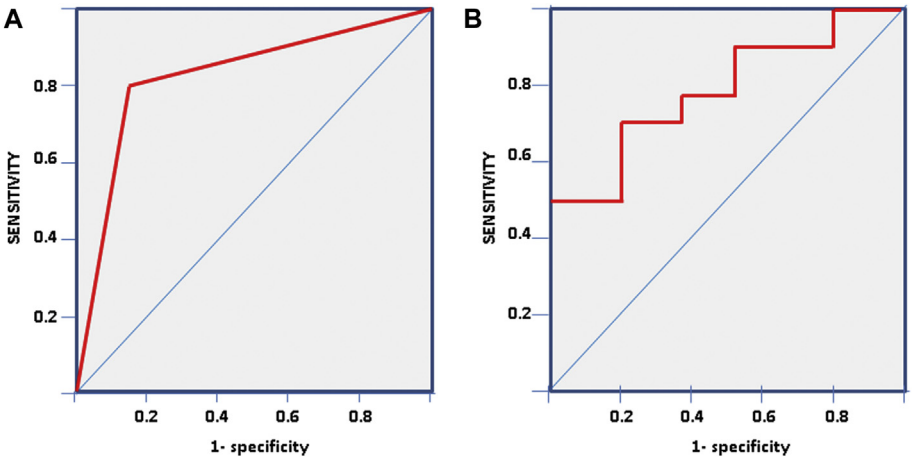

FIG 1. A, ROC for urticaria/angioedema. **B,** ROC for serum tryptase level.

HVA (anaphylaxis) with an increased SBT level, a BM assessment is usually recommended to rule out the presence of SM. The case is more complex when, despite the severity and number of reactions, SBT levels are normal (ie, <11.4 ng/mL) and no evidence of urticaria pigmentosa is present. In a recent article in 44 patients suspected of having CMDs without skin lesions and an SBT of less than 10 ng/mL, the diagnosis of CMD was not confirmed, but data about HVA were not reported.[10] In this study it was suggested that a low SBT level (<10 ng/mL) should discourage a BM examination.

Nonetheless, in the case of normal SBT levels, the correct diagnosis of CMD could be missed, with important clinical and therapeutic implications. Certainly, a predictive clinical algorithm would be helpful to select those patients who should undergo a BM examination. A previously proposed but not fully validated algorithm (Red Espanola de Mastocitosis [REMA])[16] showed good performances in identifying CMDs, but it could not be reliably applied or validated in our population because 2 of 4 parameters (presence of presyncope or syncope and tryptase levels) were already fixed as inclusion criteria.

Failing to identify a CMD in patients with normal SBT levels could possibly be related to the technical approach used. In fact, an acquisition of only 300,000 events for BM MC immunophenotyping cannot be sufficient to detect atypical MCs in patients with a very low MC burden. Moreover, in the presence of a very low BM MC burden, *KIT* D816V mutation analysis should be performed with very sensitive techniques, such as quantitative RT-PCR.

Our results highlight that the characteristics of severe HVA episodes with hypotension in the absence of urticaria/angioedema might represent the most relevant factor to identify those patients with HVA and CMDs, regardless of baseline tryptase levels. In our case series tryptase levels of 7.95 ng/mL or greater seem to be associated with the risk of SM with the highest values of sensitivity and specificity, above all in the case of severe anaphylaxis without skin symptoms.

In such patients, who are indeed very rare, a proper and adequate diagnosis should be offered without delay. In fact, an early diagnosis of CMD in patients with normal tryptase levels and severe HVA would represent a substantial advantage for several reasons. First, these patients are at high risk of severe osteoporosis, and early therapy can be immediately started to prevent vertebral fractures.[17] Second, some fatal sting reactions in patients with SM and HVA have been described after stopping venom immunotherapy,[18,19] and therefore a correct diagnosis is essential to recommend lifelong treatment. As a speculative aspect, we can hypothesize that patients with no skin involvement, normal SBT levels, and anaphylaxis with ascertained hypotension and loss of consciousness caused by Hymenoptera stings should undergo a BM examination to detect a possible CMD. In those patients with very low MC burden, the BM study should be necessarily performed in reference centers for MC diseases by using highly sensitive and adequate techniques.

Clinical implications: Anaphylaxis with hypotension in patients with HVA without urticaria/angioedema or typical skin lesions might represent a significant factor for identifying CMDs, regardless of serum tryptase levels.

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