

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.

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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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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* (Anallergo 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 10⁶ cells per sample using a FACSCanto cytometer (BD Biosciences, San Jose, Calif) and up to 6 × 10⁶ 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

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