Original Article

Anaphylactic Reactions After Discontinuation of Hymenoptera Venom Immunotherapy: A Clonal Mast Cell Disorder Should Be Suspected

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What is already known about this topic? There are no data on the presence and prevalence of mastocytosis in patients who experience severe reactions from Hymenoptera stings after the termination of an effective venom immunotherapy (VIT) course.

What does this article add to our knowledge? Mastocytosis should be suspected in those patients with Hymenoptera allergy who experience severe reactions and anaphylaxis after stopping VIT that had been previously protective.

How does this study impact current management guidelines? A proper diagnostic procedure for mastocytosis should be considered in patients developing severe reactions at re-sting after VIT discontinuation. VIT should therefore be continued lifelong in subjects with mastocytosis and venom allergy.

BACKGROUND: Up to 75% of patients with severe anaphylactic reactions after Hymenoptera sting are at risk of further severe reactions if re-stung. Venom immunotherapy (VIT) is highly effective in protecting individuals with ascertained Hymenoptera venom allergy (HVA) and previous severe reactions. After a 3- to 5-year VIT course, most patients remain protected after VIT discontinuation. Otherwise, a lifelong treatment should be considered in high-risk patients (eg, in mastocytosis). Several case reports evidenced that patients with mastocytosis and HVA, although protected during VIT, can re-experience severe and sometimes fatal reactions after VIT discontinuation.

OBJECTIVE: To evaluate whether patients who lost protection after VIT discontinuation may suffer from clonal mast cell disorders.

METHODS: The survey describes the characteristics of patients who received a full course of VIT for previous severe reactions and who experienced another severe reaction at re-sting after VIT discontinuation. Those with a Red Española de Mastocitosis score of 2 or more or a serum basal tryptase level of more than 25 ng/mL underwent a hematological workup (bone marrow biopsy, KIT mutation, expression of aberrant CD25) and/or skin biopsy.

RESULTS: Nineteen patients (mean age, 56.3 years; 89.5% males) were evaluated. All of them had received at least 4 years of VIT and were protected. After VIT discontinuation they were re-stung and developed, in all but 1 case, severe anaphylactic reactions (12 with loss of consciousness, in the absence of urticaria/angioedema). Eighteen patients (94.7%) had a clonal mast cell disorder, 8 of them with normal tryptase.

CONCLUSIONS: Looking at this selected population, we suggest that mastocytosis should be considered in patients developing severe reactions at re-sting after VIT discontinuation and, as a speculation, patients with mastocytosis and HVA

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Abbreviations used
BM- bone marrow
BMM- bone marrow mastocytosis
CM- cutaneous mastocytosis
CMD- clonal mast cell disorder
HVA- Hymenoptera venom allergy
ISM- indolent systemic mastocytosis
MC- mast cell
SBT- serum basal tryptase
SM- systemic mastocytosis
VIT- venom immunotherapy

should be VIT-treated lifelong. **© 2017 Published by Elsevier Inc. on behalf of the American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2017;■:■-■)**

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Systemic mastocytosis (SM) is a heterogeneous hematological disease characterized by the proliferation and accumulation of mast cells (MCs) in various tissues, with a preferential localization in bone marrow (BM) and skin.[1] Mastocytosis can be defined as cutaneous (CM) when confined to the skin and as systemic (SM). The diagnosis of SM requires the presence of the major criterion (multifocal dense MC infiltrates in BM or other extracutaneous organs) plus 1 minor criterion or 3 minor criteria: abnormal morphology of extracutaneous MC (spindle-shaped cells), increased serum basal tryptase (SBT) level of more than 20 ng/mL, expression of CD25 and/or CD2 on BM MC, and detection of a KIT mutation at codon 816 in extracutaneous organs. The most common presentation of SM is indolent systemic mastocytosis (ISM), but other less frequent forms are now recognized: smouldering SM, SM with associated hematologic neoplasia, aggressive SM, MC leukemia, and MC sarcoma.[2] Of note, patients with mediator-related symptoms not fulfilling the World Health Organization (WHO) criteria for SM, but carrying KIT-mutated and/or CD25-positive clonal BM MC, are considered as having a clonal mast cell disorder (CMD) defined also as (mono)clonal mast cell activation syndrome.[3,4]

A preferential association between Hymenoptera venom allergy (HVA) and SM is now recognized,[4-7] probably because Hymenoptera sting can be a trigger for anaphylactic reactions in patients with mastocytosis, who are "per se" prone to release mediators. HVA is associated in many cases to ISM without skin involvement, which is also defined as BM mastocytosis (BMM), a provisional SM variant still unrecognized by the WHO classification.[1,2,4,7-9]

Venom immunotherapy (VIT) is effective in protecting most patients with HVA, even after its discontinuation. Nonetheless, we observed that a small proportion of patients, with previous severe reactions, lost the acquired protection when VIT was discontinued. Thus, we studied these patients to assess whether an unrecognized MC-related disorder was present.

METHODS

Patients and diagnosis

Patients referred to the Verona Multidisciplinary Outpatients Clinics for Mastocytosis from a nationwide network of allergists between January 2009 and May 2014 were included in this survey. All had been diagnosed with HVA according to guidelines[10] and underwent a 4- to 11-year course of VIT for their previous severe reactions. We considered for the analysis patients who experienced severe reactions at re-sting after VIT discontinuation. None of them had a previous ascertained diagnosis of mastocytosis. A basal tryptase evaluation was not performed before VIT because before 2007 the test was not available routinely. All patients provided their informed consent for allergy and hematological assessments, as per the standard diagnostic procedures adopted in our hospital. The institutional ethical committee was notified, but no formal approval was required for this observational, noninterventional study; all procedures were part of the standard of care.

At baseline, before VIT prescription, patients underwent the standard workup for the diagnosis of HVA.[10] Skin test was performed with commercial extracts of *Polistes dominulus*, *Vespa crabro* (Anallergo Diagnostics, Florence, Italy), *Vespula* species, and honeybee (Stellergenes Greer, Antony, France). Four venom concentrations (100 μg/mL for prick test and 0.02 mL of 0.01, 0.1, and 1.0 μg/mL for intradermal test) were used, plus a negative control (NaCl 0.9%) and a positive control (histamine dihydrochloride 10 mg/mL). Serum-specific IgE for the same venoms and serum tryptase were always assayed by the ImmunoCAP test (Thermo Fischer Scientific Inc, Uppsala, Sweden) at least 2 weeks after an acute episode. The REMA score, proposed by the Red Española de Mastocitosis and based on 4 clinical/laboratory parameters (male sex, SBT $>$ 25 ng/mL, presence of hypotension, and absence of angioedema/urticaria), was applied to predict the risk of CMD.[4]

All patients who experienced a systemic reaction at re-sting after VIT discontinuation with a REMA score of 2 or more or an SBT of more than 25 ng/mL were assessed for the presence of SM according to the diagnostic algorithm proposed by the European Competence Network on Mastocytosis.[4,11] Thus, they underwent a dermatological evaluation to identify skin lesions, and a skin biopsy of suspected lesions was performed. A complete BM evaluation, including smear and biopsy, detection of D816V mutation of KIT, and flow cytometry,[12-14] was also carried out. For the analysis of MC immunophenotype, we used a highly sensitive multiparameter flow cytometry approach, as previously reported. Briefly, BM cells were stained using a combination of 5 monoclonal antibodies, CD45, CD117, CD34, CD25, and CD2, and up to 6×10^6 cells per sample were acquired using a FACSCanto cytometer (BD, Becton Dickinson, Milan, Italy). D816V KIT mutation was detected on total RNA from mononuclear cell fractions of the BM samples.[15] In the 2 patients who refused BM study, the D816V KIT mutation was searched for g-DNA from peripheral blood samples according to the method proposed by Kristensen et al.[15] The diagnosis of SM was made according to current WHO guidelines.[1]

RESULTS

Nineteen patients (2 females, age range, 41-83 years) were evaluated according to the aforementioned criteria (Table I). All of them had been previously diagnosed with HVA (6 *Polistes dominulus*, 12 *Vespula crabro*, and 1 honeybee) and have had a systemic reaction at first sting (grade IV according to Mueller in all but 1 case), most of them with loss of consciousness. All received a VIT course (range, 4-17 years), during which 13 patients were re-stung by the same causing insect and had only local reaction or large local reactions. One to 11 years after the VIT discontinuation, all patients had a field re-sting with a Mueller