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CASE REPORT

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Unraveling wasp sensitization in a patient with systemic mastocytosis by CAP-inhibition assay

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Abstract
Systemic mastocytosis (SM) is a clonal mast cell disorder that can lead to potentially severe anaphylactic reactions. Hymenoptera sting is one of the most frequent triggers of anaphylaxis in these patients, and diagnosis of indolent SM (ISM) without skin involvement (ISMs) is not rare. In this subgroup of patients, venom immunotherapy (VIT) is an effective treatment decreasing subsequent systemic reactions, and lifelong administration is recommended. An individualized diagnosis is necessary to offer the most adequate VIT, and molecular diagnosis (MD) may be useful to discriminate between primary sensitization and cross-reactivity. Nevertheless, other techniques such as ImmunoCAP inhibition assays may be necessary to identify the genuine sensitization to offer the most suitable VIT. We present a male patient with an anaphylactic reaction following several wasp stings. The patient was diagnosed with ISM, and allergy to both *Polistes dominula* and *Vespula sp* venom was confirmed. In this scenario, MD did not discriminate between a genuine double sensitization and venom cross-reactivity between both vespids. Thus, CAP-inhibition assay was performed. This case indicated the importance of an accurate diagnosis of hymenoptera venom allergy (HVA). It also highlights the usefulness of CAP-inhibition assays when MD fails to distinguish between genuine double *Polistes-Vespula* sensitization and cross-reactivity.
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Introduction

Systemic mastocytosis (SM) is a clonal disorder characterized by proliferation and accumulation of mast cells (MC) in extracutaneous tissues, mostly bone marrow, with or without skin involvement. The sting of hymenoptera is one of the most frequent triggers of anaphylaxis in these patients. Performing the REMA score in these scenarios is useful for risk stratification often leading to the diagnosis of ISMs.[1-3]

Hymenoptera venom allergy (HVA) is an immunoglobulin E (IgE)-mediated reaction caused by sensitization to one or more venom allergens.[2] Diagnosis is based on a suggestive clinical history and confirmation of venom IgE sensitization either by skin tests (IDT) or by in vitro tests demonstrating sIgE to hymenoptera venom (HV).[4]

Venom immunotherapy (VIT) is a safe and effective treatment decreasing subsequent systemic reactions. Lifelong administration is recommended in SM patients. An individualized diagnosis is necessary to offer the most adequate VIT. In some cases, molecular diagnosis (MD) may be useful to discriminate between primary sensitization and cross-reactivity in patients with double-positive results to vespid venoms.[4] Nevertheless, in other cases, different techniques such as specific IgE (sIgE) and ImmunoCAP inhibition studies (CAP Inhibition) may be necessary to confirm or rule out co-sensitization to identify the most suitable venom for VIT.[3,5] Other ex vivo tests, such as basophil activation test (BAT), may also be useful but are less commonly used in daily practice due to their technical requirements.[4]

Table 1 Allergy workup. IMMUNOCAP® Thermofisher®, Sweden.

IgE	**(kU/l)**
Total IgE	104
Specific IgE	**(kUA/l)**
sIgE MUXF3-CCD (o214)	0.01
sIgE *Apis mellifera* (i1)	0.02
sIgE rApi m 1 (i208)	0.01
sIgE rApi m 2 (i214)	0.01
sIgE rApi m 3 (i215)	0.00
sIgE rApi m 5 (i216)	**0.36**
sIgE rApi m 10 (i217)	0.01
sIgE *Vespula spp* (i3)	**1.58**
sIgE rVes v 1 (i211)	**0.25**
sIgE eVes v 5 (i209)	**1.05**
sIgE *Vespa crabro* (i75)	**0.80**
sIgE *Vespa velutina* (U1223)	**1.82**
sIgE *Polistes dominula* (i77)	**3.17**
sIgE rPol d 5 (i210)	**1.82**
sIgE *Polistes spp* (i4)	**2.62**
Triptase (µg/l)	**25.3**

Case Report

We present the case of a 48-year-old male gardener with no significant past medical history. He was referred to our allergy department 6 months after suffering an anaphylactic reaction (grade 3 of European Academy of Allergy & Clinical Immunology (EAACI) classification and grade 3 of Mueller classification)[6] following several wasp stings. This incident occurred in an area where both Vespula and Polistes dominula vespids are present, but the culprit vespid could not be identified. Immediately after the sting, the patient experienced a large local reaction, followed by nasal obstruction, dyspnea, and stridor without further cutaneous involvement. However, there was complete recovery in minutes following treatment in the emergency department with oral corticoids and antihistamines. Unfortunately, vital signs of the acute reaction were missing, and no symptoms compatible with hypotension were present.

Complete allergy workout was performed using skin tests and in vitro tests (*ImmunoCAP®, Thermofisher®*, Sweden) to sIgE to whole extracts to different hymenoptera venoms. MD including sIgE to single hymenoptera venom components to identify the primary sensitizer, and sIgE to MUXF3 to rule out sensitization to cross-reactive carbohydrate determinants (CCDs) were also done. Results are summarized in Table 1.

The REMA score (Spanish Network on Mastocytosis)[7] was calculated to assess the risk of SM, yielding a score of 4, which is highly suggestive of clonality. Consequently, a bone marrow biopsy was performed, which showed atypical MC with aberrant phenotype (CD25+++, CD2++), although D816V kit mutation was not present.

Since levels of sIgE to group 5 antigens of both hymenoptera species were very similar and did not allow discriminating, the primary sensitizer CAP-inhibition assays were performed following a previously described protocol with some modifications.[8] A blood sample from the patient was collected, and the serum was stored at −20°C until use. Inhibition of the patient's serum-specific IgE was performed with commercial venom preparations (HYMNOX®, Roxall®, Spain) as inhibitors. Briefly, 50 µl aliquots of the patient's serum were incubated at 4°C overnight with 100 µl of the diluent (to obtain one-third of the diluted control serum), and 100 µl of vespid venom at a concentration of 100 µg/mL (*Vespula spp*, *Polistes dominula*, or a mixture of both venoms) in four separate test tubes. Serum sIgE determination of *Vespula spp* and *Polistes dominula* (ImmunoCAP) was run on the next day. The extent of inhibition was calculated with the following formula: [1−(inhibited sIgE/control sIgE)] ×100. Arbitrarily, inhibitions ≥ 70% were considered relevant. Homologous inhibition (blockage of venom-sIgE by the same venom) and heterologous inhibition (blockage of the venom-sIgE by the other venom) were also considered.[9,10]

Levels of sIgG4 to vespid venoms were performed at baseline and 6 months after VIT initiation. Low titers of sIgG4 against *Polistes dominula and Vespula spp.* (0.68 mg_A/L and 0.26 mg_A/L, respectively) at baseline may be interpreted as a marker of prior hymenoptera venom exposure.

Results

Intradermal tests yielded positive results for *Polistes dominula* at 0.1 µg/mL and for *Vespula spp* at 1 µg/mL.

Table 2 CAP-inhibition assays.

Tube 1. Dilution (50 µl serum + 100 µl of diluent) (diluted control 1/3)		
sIgE *Vespula spp* (i3)	0.54 kU_A/l	
sIgE *Polistes dominula* (i77)	1.25 kU_A/l	
Tube 2. *Vespula spp*. (50 µl serum + 100 µl venom of *Vespula germanica* + *vulgaris*) (10 µg of total venom)		
sIgE *Vespula spp* (i3)	0.15 kU_A/l	**72% inhibition**
sIgE Polistes dominula (i77)	0.69 kU_A/l	45% inhibition
Tubo 3. *Polistes dominula* (50 µl serum + 100 µl venom of *Polistes dominula*) (10 µg of total venom)		
sIgE *Vespula spp* (i3)	0.26 kU_A/l	52% inhibition
sIgE *Polistes dominula* (i77)	0.27 kU_A/l	**78% inhibition**
Tube 4. Mixture of *Vespula spp* + *Polistes dominula* (50 µl serum + 50 µl venom of *Vespula germanica* + *vulgaris* + 50 µl venom of *Polistes dominula*) (10 µg of total venom)		
sIgE *Vespula spp* (i3)	0.16 kU_A/l	**70% inhibition**
sIgE *Polistes dominula* (i77)	0.19 kU_A/l	**85% inhibition**

The extent of inhibition was calculated with the following formula: [1−(sIgE inhibited/sIgE control)]*100. Inhibition ≥ 70% is considered relevant.

Skin tests with *Apis mellifera* venom were negative. Basal serum tryptase was 25.3 µg/L.

sIgE determination was positive to *Polistes dominula* (3.17 kU_A/l; *rPol d 5* (i210) 1.82 kU_A/l) and *Vespula spp* (1.58 kU_A/l; *rVes v 1* (i211) 0.25 kU_A/l, *eVes v 5* (i209) 1.05 kU_A/l), as well as to *Polistes spp.* (2.62 kU_A/l), *Vespa velutina* (1.82 kU_A/l), and *Vespa crabro* (0.80 kU_A/l). Triptase levels were 25.3 µg/l, and values for *MUXF3-CCD (o214)* were negative (Table 1).

The results of the CAP inhibition assays are shown in Table 2. Incubation with isolated venoms achieved only relevant (≥ 70%) homologous inhibition. The inhibition with the combination of both venoms was superior to the inhibition with *Vespula* and *Polistes dominula* venoms separately. This meant that the patient had a genuine double sensitization to *Polistes dominula* and *Vespula spp,* and therefore would mostly benefit from a VIT with both venoms. With these findings, the patient was diagnosed with ISMs and double *Polistes dominula-Vespula* venom hypersensitivity.

Six months after VIT was started, levels of sIgG4 to both venoms were increased (3.61 mg_A/L and 3.27 mg_A/L, respectively). In spite of IgG4 not being validated as an unequivocal marker of protection, the patient experienced an accidental wasp sting while on maintenance. Fortunately, there was only a small local reaction, which indicated the acquisition of clinical tolerance.

Discussion

The patient's results demonstrated a genuine double sensitization to Polistes dominula and Vespula spp, leading to the recommendation of VIT with both venoms. We started the double VIT using the standard rush protocol alternating every month between Vespula and Polistes dominula VIT. After 6 months in the maintenance phase, the patient showed an increase in sIgG4 levels, indicating immunological activity. Also, a new vespid sting resulted only in minimal local reaction, indicating the efficacy of this treatment approach.

Conclusions

In spite of the strong association between ISMs and HVA, the former continues to be underdiagnosed. Therefore, a high degree of suspicion is required to confirm the diagnosis. In cases of anaphylactic reactions (especially without skin involvement) and high levels of basal serum tryptase, a REMA score should always be performed to assess the probability of a MC disorder.

This clinical case also highlights the importance of an accurate diagnosis of HVA and the usefulness of CAP-inhibition assays when the MD is unable to distinguish between genuine double *Polistes-Vespula* sensitization and cross-reactivity.

Conflicts of Interest

The authors declare no potential conflicts of interest with respect to research, authorship, and/or publication of this article.

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