



Venom immunotherapy in patients with mastocytosis

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Abstract

Background A clear relationship has been demonstrated between hymenoptera venom anaphylaxis (HVA) and primary mast cell activation syndromes (MCAS).

Methods This review describes the clinical and biological characteristics of patients with primary MCAS who present with HVA, the diagnostic tests that have demonstrated to be useful, and the safety and effectiveness of venom immunotherapy (VIT) in these individuals.

Results Patients presenting with dizziness or hypotension and without skin involvement after an insect sting are at an increased risk of presenting clonal mast cells (MCs). They usually have low MC burden and highly sensitive techniques are mandatory to reach a final assessment. Skin testing and determinations of specific IgE are useful in detecting patients who are candidates for VIT, which has proved to be safe and effective.

Conclusions Patients with primary MCAS and IgE-mediated HVA are candidates to receive VIT. Their management does not differ much from general population with the exception of VIT duration.

Keywords Anaphylaxis · Clonal · Hymenoptera · Mast cell activation syndromes · Sting

Abbreviations

ASO-qPCR	Allele-specific oligonucleotide quantitative polymerase chain reaction
BAT	Basophil activation test
BM	Bone marrow
FACS	Fluorescence-activated cell sorting
HVA	Hymenoptera venom anaphylaxis
ISM	Indolent systemic mastocytosis
ISM _s	Indolent systemic mastocytosis without skin involvement
MC	Mast cell
MCAS	Mast cell activation syndrome
MMAS	Monoclonal mast cell activation syndrome
REMA	Red española de mastocitosis (Spanish network on mastocytosis)
SAR	Systemic adverse reactions
SC	Sting challenge
SM	Systemic mastocytosis
UP	Urticaria pigmentosa
VIT	Venom immunotherapy
WHO	World health organization

Introduction

Patients with primary mast cell (MC) activation syndromes (MCAS) [1, 2]—which include systemic mastocytosis (SM) and clonal or monoclonal MCAS (MMAS) (Table 1)—often present with anaphylaxis [3]. Moreover, a clear relationship has been demonstrated between hymenoptera venom anaphylaxis (HVA) and primary MCAS [4]; in fact, hymenoptera is the first cause of anaphylaxis in patients with SM in the absence of skin lesions compatible with urticaria pigmentosa (UP) [5]. An early study by Bonadonna et al.

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Table 1 Classification of mast cell activation syndromes (MCAS). (Adapted from reference Valent et al. [2])

Types of MCAS	Diagnostic characteristics
<i>Primary</i>	
Systemic mastocytosis	One major criterion and one minor criterion or at least three minor WHO criteria ^a are required
Clonal or monoclonal MCAS	Only 1 or 2 WHO minor SM criteria ^a are fulfilled
<i>Secondary</i>	IgE-mediated allergy or other conditions that can activate MCs exist in the absence of neoplastic MCs or <i>KIT</i> mutation
<i>Idiopathic</i>	MCAS criteria are met but neither primary nor secondary causes of mast cell activation are detected

BM bone marrow, *MC* mast cell, *MCAS* mast cell activation syndrome, *SM* systemic mastocytosis, *WHO* World Health Organization

^aDiagnostic criteria of mastocytosis, according to the WHO. Major criteria: multifocal dense infiltrates of MCs (>15 MCs aggregating) detected in BM sections and/or other extracutaneous organ(s) by tryptase-immunohistochemistry or other MC-associated stains. Minor criteria: (i) more than 25% of MC are spindle-shaped in MC infiltrates detected in BM sections or other extracutaneous tissue sections OR >25% atypical MC detected in BM smears, (ii) detection of a *KIT* point mutation at codon 816 in BM MCs or other extracutaneous organ(s), (iii) expression of CD25 and/or CD2 on mast cells in BM MCs, blood or other extracutaneous tissues, and (iv) total serum baseline tryptase concentration persistently >20 ng/ml (in case of an systemic mastocytosis with associated clonal hematological non-mast cell lineage disease, this criteria is not valid)

reported an overall frequency of clonal MC disorders in 7.9% [6] of the patients suffering from life-threatening reactions after bee and/or wasp stings, but a more recent report has suggested that this percentage might be as high as 26% [7]. Venom immunotherapy (VIT) is the only treatment that can prevent further systemic IgE-mediated reactions in venom allergic patients, and it has proven to be a safe and effective procedure [8]. Here, we review the clinical and biological characteristics of patients with primary MCAS who present with HVA, the diagnostic tests that have been demonstrated to be useful, and the safety and effectiveness of VIT in this subset of patients.

Clinical and biological characteristics of primary MCAS presenting with hymenoptera venom anaphylaxis

Patients with indolent SM in the absence of UP (ISMs-) and MMAS associated with anaphylaxis exclusively triggered by insect bite or sting have been shown to display unique clinical, biological and molecular features vs. other subtypes of SM [3, 4, 9]. These distinctive features include a clear male prevalence with a male-to-female ratio of 8:2, a typical pattern of MC-mediator release symptoms during anaphylaxis characterized by cardiovascular symptoms in the absence of pruritus, urticaria and angioedema, and a low MC tumor load as reflected by relatively lower serum baseline tryptase levels and more frequent coexistence of normal and neoplastic bone marrow (BM) MCs compared to other ISMs-cases, together with the absence of compact BM MC aggregates in more than half of the cases [9].

Of note, the Spanish Network on Mastocytosis (REMA) score, a predictive tool for ISMs- and MMAS, has shown a specificity and sensitivity of 67 and 92%, respectively, for the identification of clonal MCs in a group of 155 patients suffering from systemic MC activation symptoms exclusively triggered by insects who were referred to the REMA [3, 9]. The particularly high sensitivity of the REMA score in this clinical setting supports its utility as a screening method to

select those patients who are candidates for a BM study.

From a molecular point of view, the D816V *KIT* mutation is detected in more than 95% of the patients provided that highly sensitive diagnostic methods are used. Interestingly, this mutation is typically restricted to the BM MC compartment, which indicates that the probability of progression to advanced forms of the disease is an exceptional event in patients with ISMs- in whom anaphylaxis is triggered only by insects [9].

Assessment of mast cell clonality in patients with suspected primary MCAS

The diagnostic study of SM and MMAS is based on the demonstration of neoplastic (clonal) MCs in the BM together with the measurement of serum baseline tryptase levels. According to the World Health Organization (WHO), there are five diagnostic criteria (one major criterion and four minor criteria) for the diagnosis of SM [10]. The major criterion consists of the demonstration of multifocal compact aggregates of >15 MCs in BM sections, whereas the minor criteria include the following: (1) >25% of BM MCs with an abnormal morphology, (2) aberrant expression of CD25 and/or CD2 by BM MCs, (3) detection of a mutation at codon 816 of *KIT*, and (4) baseline serum tryptase >20 µg/l. When the major criterion and at least one of the four minor criteria or, when three minor criteria in the absence of the major criterion are present, the diagnosis of SM is established; in turn, MMAS is defined by the existence of 1–2 markers of MC clonality (i.e., CD25 expression and/or codon 816 *KIT* mutation) without other criteria [11, 12].

The low BM MC burden that typically accompanies ISMs- and MMAS frequently translates into a substantial proportion of patients lacking compact BM MC aggregates and showing serum tryptase levels below 20 µg/l. Therefore, highly sensitive diagnostic methods are mandatory for the study of patients presenting with systemic MC-mediator release symptoms in the absence of UP [13]. These methods include multiparameter flow cytometry immunophenotyping for the identification and characterization of MCs present in

the BM at very low frequencies (e.g., $<10^{-4}$ to $<10^{-5}$) [14], and molecular assessment of the D816V KIT mutation using allele-specific oligonucleotide real time quantitative PCR (ASO-qPCR) method [15]. Of note, the sensitivity to detect the D816V KIT mutation can be improved when molecular studies are performed in fluorescence-activated cell sorting (FACS)-purified BM MCs, as repeatedly reported elsewhere [9, 16].

Indications for immunotherapy and diagnostic tests

As for general population, VIT should be recommended upon demonstration of IgE-mediated allergy for patients with primary MCAS who present with systemic reactions involving organs other than the skin, in those who suffer extensive skin reactions in the context of high risk of field exposure and/or impaired quality of life and, more rarely, in some patients with recurrent, troublesome local large reactions [8].

Diagnostic tests

Only those patients who are candidates to VIT should undergo diagnostic tests [17]. The following assays may help in the identification of the culprit insect in primary MCAS patients:

- Skin tests (prick tests and intradermal tests) are the gold standard for diagnosis of IgE-mediated allergy [8] and can be carried out safely in patients with clonal MC disorders [18]. In order to avoid false-negative results, skin tests should be performed, at least, 2 weeks after the last insect sting [8].
- Serum allergen-specific IgE antibody measurements using whole extracts have demonstrated lower sensitivity than skin tests; despite this, it is recommended to perform both diagnostic assays in the allergy work-up of patients with primary MCAS, as it is also advised for the general population [8, 17]. It is well known that patients with mastocytosis have lower levels of total and specific serum IgE vs. the general population [19]; thus, it has been proposed to use a lowered cut-off point of 0.1 kU/l in patients with clonal MCs [9]. In this regard, component-based determination of serum specific IgE antibodies to hymenoptera venom allergens has proven to be a useful tool for detecting truly double sensitization [8], higher risk sensitization profiles [20, 21], and also for the demonstration of an IgE-mediated mechanism of allergy in a subset of patients with primary MCAS associated with hymenoptera-induced anaphylaxis with both negative skin tests and serum allergen-specific IgE antibodies [9]. CAP inhibition assays may also help to distinguish between double sensitization and cross-reactivity but is not widely available and the results are, sometimes, difficult to interpret [17].

- Basophil activation test (BAT) has shown contradictory results in patients with primary MCAS: while some reports suggest a potential utility of BAT in patients with MC disorders and negative venom-specific IgE and skin test results [22], other studies have not confirmed the same results [23]. It has been recently suggested that an adequate selection of the molecule used for the identification of basophils (CCR3 vs. IL3 receptor—CD123—in conjunction with HLA-DR or CD45) might be determinant in the effectiveness of BAT when applied to mastocytosis patients [24]. Altogether, these observations suggest that BAT might be considered as a complementary diagnostic tool in patients with clonal MCs who are negative for skin tests or sIgE or display low total IgE levels.

VIT extracts and protocols

The variety of extracts used in patients with clonal MC disorders do not differ from those employed in the general population, and their safety profile do not seem to be different either. Purified venom preparations have lower frequency of adverse events than nonpurified aqueous preparations [17], and depot extracts appear to be associated with better tolerability than aqueous preparations; however, these findings might be biased by the slower build-up phase normally used with depot preparations [8].

Premedication of VIT is generally not needed unless adverse reactions occur, although many patients might be already on daily anti-mediator drugs such as disodium cromoglycate or antihistamines.

Different protocols have been proposed to reach the maintenance protective dose of 100 mcg, considered the gold standard as in general population. In unprotected patients, this dose should be raised to 200 mcg or higher, if needed [8, 17, 25]. Traditionally, conventional protocols had been recommended in patients with primary MCAS since they had proven their safety in large series of patients [25], and it was also known that in patients with elevated baseline tryptase the risk of a reaction with an ultrarush protocol was almost twice compared with a rush schedule [26]. Nevertheless, recent studies using ultrarush protocols in patients with clonal MCs have shown to be safe as well [27, 28].

Safety of VIT

Vespula, *Polistes*, *Apis mellifera* and imported fire ant immunotherapy is usually well tolerated by the majority of patients with primary MCAS [4, 25]. The overall frequency of systemic side effects during VIT is estimated at 24% [29]; in a study with 29 patients with clonal MCs, 6 of them (29%) experienced systemic adverse reactions (SAR) during VIT [30]. A larger study with 84 patients reported 4.7% of SAR, and authors found a slightly higher number of adverse reactions

with the rush-modified induction regimen compared to the conventional protocol. Though there was no statistical significance, the authors suggested a less aggressive induction protocol in patients with clonal MCs [31]. In different studies carried out in general population, VIT shows a spectrum of SAR that range from 8–20% [8]. Therefore, regarding published data, systemic adverse reactions in mastocytosis patients do not seem to differ much from the percentage of adverse reactions in general population.

Concerning safety of VIT depending on the type of venom, Vega-Castro et al. [28] found, in 9 patients with primary MCAS who were receiving VIT, SAR only in the patients treated with bee VIT (3/9), which is in accordance with results found in other studies from South Europe [31] and Israel [32], and contrasts with data from Central Europe that reported SAR in mastocytosis patients mainly treated with vespid VIT [26]. Based on their findings, the author proposes to start on disodium cromoglycate in patients with a high suspicion of primary MCAS prior to the onset of VIT, especially when they are going to receive bee VIT [28].

Effectiveness of VIT

Effectiveness, understood as the decrease or demise of the patient's symptoms once they confront the allergen naturally during maintenance of VIT, or after discontinuation, is reached in around 77 to 84% of the general population treated with honeybee venom, and in 91 to 96% of patients receiving vespid venom [8]. In a review of 111 patients with mastocytosis and HVA the rate of efficacy of VIT after re-sting was 74% [25]. In a study with 84 Italian and Spanish mastocytosis patients VIT showed full protection in 86% of the patients who were field re-stung. In those patients who were not fully protected after a sting, an increase of the maintenance dose to 200 mcg venom was safely carried out; thus, in unprotected cases, an increase of the maintenance dose can be reasonably recommended, as in the general population [8, 31]. Similar results of VIT efficacy (83.6%) were observed in a large multicenter study including patients with mastocytosis in the skin and patients with elevated baseline serum tryptase [26].

Additionally, it has been recently published that elevated specific IgG4 levels may be a useful biomarker for assessing the efficacy of VIT in patients with clonal MCs, as they correlate with protection from re-stings [33]. Nevertheless, primary MCAS patients, even when the evolution of VIT is adequate, should be advised to permanently carry epinephrine autoinjectors because of their higher risk of sting adverse reactions and the possibility of developing a different insect venom allergy [34].

Sting challenge (SC) is still the gold standard method to assess the effectiveness of VIT [8] and can also improve the health-related quality of life in bee and vespid venom allergic patients [35]. Nonetheless,

performing sting challenges in primary MCAS patients is a controversial topic. Rueff et al. found 11.3% of systemic reactions after hospital sting challenge test in patients with mastocytosis in the skin or basal tryptase >20 ng/mL compared to 6.3% in general hymenopteran allergic patients [26]. Other studies have reported a higher (23.9%) rate of systemic reactions after sting challenge in patients with mastocytosis [29], which forces to be cautious when performing SC in these patients. Even so, it is important to identify primary MCAS patients who are not protected with conventional doses of VIT in order to increase the maintenance dosage and achieve efficacy [36].

Duration of VIT

Nowadays, there are no available data to evaluate long-term efficacy of VIT in patients with primary MCAS. Fatalities have been communicated in these patients after stopping VIT [37]. Bonadonna et al. recently reported a group of 19 patients who were field re-stung a mean time of 5 years after VIT discontinuation and presented SAR. Surprisingly, 18/19 (94.7%) had clonal MCs [38]. This evidence strongly supports the recommendation of extending VIT over the standard duration in patients with primary MCAS, or preferably life-long.

Within the normal population, in venom allergic patients who need VIT for longer periods than usual, it has shown to be safe and protective a 3- to 4-month extended interval after the 5th year of VIT in order to improve long-term compliance [39, 40]. In patients with primary MCAS, maintenance doses every 4–6 weeks, and every 3 months after the third year of treatment, have proven to be safe and effective [31]. Although there are no data regarding efficacy and safety after the 5th year of VIT in patients with clonal MCs, it seems reasonable to maintain the same suggestion as in general population.

Conclusions

Patients with anaphylactic reactions to hymenoptera venom presenting with cardiovascular symptoms such as dizziness or hypotension, and in the absence of pruritus, urticaria and angioedema, are at an increased risk for presenting primary MCAS as the underlying diagnosis. They usually have low MC burden and may show baseline tryptase levels below 20 µg/L. Consequently, highly sensitive diagnostic methods are mandatory to reach a final assessment. As for general population, VIT should be recommended when IgE-mediated allergy is demonstrated. The management of their allergy does not differ generally from that of overall community, and VIT has proven to be safe and effective. Unlike the general population, immunotherapy should be recommended long-life or, at least, extend it over the standard duration.

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