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Venom immunotherapy in patients with mastocytosis and hymenoptera venom anaphylaxis

Systemic mastocytosis (SM) is typically suspected in patients with cutaneous mastocytosis (CM). In recent years, the presence of clonal mast cells (MCs) in a subset of patients with systemic symptoms associated with MC activation in the absence of CM has been reported and termed monoclonal MC activation syndromes or clonal systemic MC activation syndromes. In these cases, bone marrow (BM) MC numbers are usually lower than in SM with CM, there are no detectable BM MC aggregates, and serum baseline tryptase is often $<20 \mu\text{g/l}$; thus, diagnosis of SM in these patients should be based on careful evaluation of other minor WHO criteria for SM in reference centers, where highly sensitive techniques for immunophenotypic analysis and investigation of *KIT* mutations on fluorescence-activated cell sorter-purified BM MCs are routinely performed. The prevalence of hymenoptera venom anaphylaxis (HVA) among SM patients is higher than among the normal population and it has been reported to be approximately 5%. In SM patients with IgE-mediated HVA, venom immunotherapy is safe and effective and it should be prescribed lifelong. Severe adverse reactions to hymenoptera stings or venom immunotherapy have been associated with increased serum baseline tryptase; however, presence of clonal MC has not been ruled out in most reports and thus both SM and clonal MC activation syndrome might be underdiagnosed in such patients. In fact, clonal BM MC appears to be a relevant risk factor for both HVA and severe reactions to venom immunotherapy, while the increase in serum baseline tryptase by itself should be considered as a powerful surrogate marker for anaphylaxis. The Spanish Network on Mastocytosis has developed a scoring system based on patient gender, the clinical symptoms observed during anaphylaxis and serum baseline tryptase to predict for the presence of both MC clonality and SM among individuals who suffer from anaphylaxis.

KEYWORDS: clonal mast cell activation syndrome ■ hymenoptera venom anaphylaxis ■ mastocytosis ■ serum baseline tryptase ■ venom immunotherapy

Since the first report in 1925 of successful desensitization of a bee allergic patient using an extract from bee venom sacs [1], many other cases of successful venom immunotherapy (VIT) have been reported. Initially, whole-body extracts were used for this purpose; however, in the 1960s, serious doubts about the efficacy of whole-body extracts arose following reports of fatalities occurring after sting [2]. As a consequence, commercial venoms became available in 1979 whose efficacy and safety was further documented [3,4]; currently, allergen vaccine remains the standard treatment for hymenoptera venom anaphylaxis (HVA) as recommended by several guidelines [5–7].

The importance of venoms as an allergen source has been widely described [8]. Insect venom allergens are proteins of 10–50 kDa containing 100–400 amino acid residues. Honeybee venom has four allergens of known sequences: phospholipase A_2 , hyaluronidase, acid phosphatase and the cytolytic peptide mellitin. By contrast, vespid venoms contain antigen 5, hyaluronidase and phospholipase A_1 as their major allergens.

Furthermore, several other relevant allergens in hymenoptera venoms have been described in recent years (e.g., dipeptidylpeptidase in honeybee and vespa venom, and the serine protease in bee venom [9]). On top of all these allergens, hymenoptera venoms also contain vasoactive amines (e.g., histamine, dopamine or norepinephrine), acetylcholine and kinins, which might also elicit the symptoms experienced after a sting, independently of allergen sensitization [8]. VIT provides efficient protection against HVA in patients at risk. Distinct mechanisms, involving different cell populations, play a key role in the protective mechanisms and processes. Tregs prevent sensitization to allergens in allergy patients through T-cell responses to venom that produce cytokines such as IL-4 and IL-13, which lead to the production of IgE. VIT seems to modify this T-cell phenotype from a Th2 type (IL-4 and IL-5 production) towards either a Th1 type (IFN- γ production) or a Treg phenotype (IL-10 expression and IgG $_4$ production) [10]. This may explain the decrease of venom-induced histamine and leukotriene release by peripheral blood

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leukocytes [11]. Besides, an increase in monocytic production of IL-12 and TNF- α might also be involved in the development of tolerance [12].

Fatal anaphylaxis in mastocytosis after hymenoptera sting was first reported in 1979 in a patient with indolent systemic mastocytosis (ISM) who died shortly after a bee sting [13]. Although in this report Brouet mentioned that the association of mastocytosis and insect sting might have been merely coincidental, he also suggested that in some cases, an offending agent such as a bee sting might precipitate mast cell (MC) degranulation in systemic mastocytosis (SM) [13]. Since then, many other cases have reported HVA in patients with mastocytosis. At first, it was mainly described in patients with SM and clinical hallmarks of urticaria pigmentosa [14–19], but later on, also in SM in the absence of skin lesions [20–27]. More recently, HVA has been reported in patients who show clinical symptoms of MC activation and lack skin lesions but carry clonal MCs without fulfilling the diagnostic criteria for SM [28–32]. In parallel, studies reporting VIT administration in such SM patients, have also been documented [14,15,17,18,20,21,25,33–35]. **Overall, data suggest that patients with mastocytosis and HVA are both at risk of severe anaphylaxis [23,31] and at risk for systemic or life-threatening reactions during VIT [18,20,21,36].**

In this article, we provide recommendations on the use of VIT in patients with hymenoptera sting anaphylaxis and mastocytosis (or other clonal MC syndromes).

Mastocytosis & other clonal mast cell activation syndromes

■ Mast cell

Mast cells are a key structural and functional component of the immune system and they are distributed throughout the human body, mainly in the vicinity of blood vessels. These cells play a key role in inflammation and they are the major effector cells in allergic reactions, including anaphylaxis. MCs contain an array of biologically active mediators in their granules that are preformed and stored. Aggregation of high-affinity Fc receptors for IgE (Fc ϵ RI) on the MC surface elicits the release of preformed granule-associated inflammatory mediators in IgE-sensitized MCs [37–39]. In addition, release of MC mediators can be elicited through Fc γ receptors [40–42], complement proteins [37–39], or aggregated IgG and C3a [43]. Moreover, MCs express Toll-like receptor (TLR)4 and TLR2, CD48 and complement receptor 1 (CD35) [44–47], which can activate MCs in the absence of other immunological signaling [48].

■ Mastocytosis

Mastocytosis includes a heterogeneous group of disorders characterized by an abnormal accumulation of low numbers of MCs, in one or multiple tissues. Patients with mastocytosis have symptoms that are mainly related to the tissue response, to the release of mediators by MCs, and also in aggressive forms, to the MC burden.

As in normal MCs, clonal MCs from patients with SM also express cell-surface antigens related to MC activation including the stem cell factor receptor (c-kit or CD117), Fc ϵ RI, IgG receptors (Fc γ R), LAMP molecules such as CD63 and complement-related receptors [39], among others. Bone marrow (BM) MCs from SM typically exhibit unique immunophenotypical features, from which the most relevant is the expression of the IL-2 high-affinity receptor α -chain (CD25) with or without coexpression of the costimulatory molecule CD2 [49]; this aberrant pattern has been included as a minor diagnostic criterion for SM [50,51]. In addition to CD25 and CD2 expression, BM MCs from SM commonly show other aberrancies such as overexpression of the CD63 [52], CD69 [53] and the ectoenzyme E-NPP3 (CD203c) [54], activation-associated molecules and several complement-related cell surface proteins including CD11c, CD35, CD59 and CD88 [55]; by contrast, membrane expression of c-kit (CD117) is abnormally downregulated [39,56]. In line with this, it should be noted that MCs from most ISM patients, with or without skin lesions, carry an activated immunophenotype characterized by overexpression of the activation-associated molecules mentioned previously [57].

■ WHO classification of mastocytosis & diagnostic criteria

In 2008, an international consensus conference defined the classification and diagnostic criteria for systemic mastocytosis as summarized in Box 1 [51].

■ Diagnosis of mastocytosis

Systemic mastocytosis with skin involvement

Systemic mastocytosis can be easily suspected in patients with skin involvement and its diagnosis should be based on the combined usage of clinical and laboratory data including serum baseline tryptase (sBT) levels, together with a complete BM study following the established methods and criteria for morphology [58], histology and immunohistochemistry [59,60], flow cytometry [49,61] and *KIT* mutational analyses [62,63].

Box 1. WHO classification of mastocytosis and diagnostic criteria.**Classification**

- Cutaneous mastocytosis:
 - Urticaria pigmentosa/maculopapular cutaneous mastocytosis
 - Diffuse cutaneous mastocytosis
 - Solitary mastocytoma of skin
 - Mast cell sarcoma
- Indolent systemic mastocytosis
- Systemic mastocytosis with associated clonal hematological non-mast-cell lineage disease
- Aggressive systemic mastocytosis
- Mast cell leukemia
- Mast cell sarcoma
- Extracutaneous mastocytoma

Diagnostic criteria for systemic mastocytosis

- Major criteria:
 - Presence of multifocal, dense infiltrates of mast cells (≥ 15 mast cells in aggregates) detected in sections of bone marrow and/or other extracutaneous organ(s)
 - Confirmation of mast cell lineage by tryptase immunohistochemistry or other special stains
- Minor criteria:
 - Identification of $>25\%$ mast cells, spindle-shaped or morphologically atypical, in biopsy sections of bone marrow or other extracutaneous organs
 - Detection of the D816V *KIT* mutation in bone marrow, blood or other extracutaneous organs
 - Aberrant coexpression of CD25 and/or CD2 by bone marrow mast cells
 - Serum tryptase levels persistently increased over $20 \mu\text{g/l}$
- Either all of the major and one minor criteria or at least three minor criteria are currently required for the diagnosis of systemic mastocytosis

Data taken with permission from [51].

Systemic mastocytosis without skin lesions & other clonal mast cell activation syndromes

In recent years, patients presenting with systemic symptoms attributable to MC activation (MCA) in the absence of mastocytosis have been reported [64,65]; such MCA syndromes (MCAS) are characterized by a number of recurrent and systemic symptoms, such as generalized pruritus, hives, flushing, tachycardia, abdominal pain, diarrhea, respiratory compromise and/or syncope or near-syncope episodes. In addition, the presence of clonal MCs in a subset of patients with 'idiopathic' anaphylaxis in the absence of mastocytosis in the skin have been reported [29,30] and termed clonal MCAD (c-MCAD) [30,31,66] or monoclonal MCAS (MMAS) [28,32]. In a recent review [64], these syndromes were renamed MCAS and they should be clearly distinguished from other allergic and nonallergy diseases (e.g., asthma, rhinitis, conjunctivitis, hypersensitivity diseases or irritable bowel disease). Diseases associated with MCA have been classified as primary (e.g., mastocytosis and MMAS), secondary (e.g., allergic disorders and inflammatory disorders, among others) and idiopathic (e.g., anaphylaxis, angioedema, urticaria and MCAS) [64]. For this reason, an efficient criteria to select those patients with MCAS who show a high probability for

having a clonal MC disorder (mainly SM without skin involvement) are warranted. Since in such cases whereby BM MC numbers are usually lower (i.e., $<10^3$) than in SM with skin lesions [30], BM MC aggregates (considered the only major criterion by the WHO [51]) are absent in 33% of the cases [30] and sBT levels are $<20 \mu\text{g/l}$ in 37% of the cases [30], diagnosis of SM in such cases should be based on careful evaluation of the other three remaining minor WHO diagnostic criteria for SM; this includes detailed cytological analysis of BM smears, flow cytometry immunophenotyping using specific gating strategies for detecting MC present at low frequencies in BM aspirate samples [49,57,61,67] and detection of *KIT* mutation in highly purified BM MC [30,63].

Hymenoptera venom anaphylaxis in mastocytosis & other clonal mast cell syndromes

■ Prevalence

The prevalence of anaphylaxis in the general population is estimated to be approximately between one and three cases to 10.5 cases per 100,000 individuals a year [68–71]. As confirmed in a large series of patients, anaphylaxis is a frequent finding among the mastocytosis population [23,72], as well as in patients diagnosed with MMAS [22,31] or clonal MCAS [30]. In turn, it has also been

reported that among patients with idiopathic anaphylaxis, some harbor an activating codon 816 *KIT* mutation in BM MCs [29]. Such cases display a common activating exon 17 *KIT* mutation in their MCs; such activating mutation(s) may result in a hyper-responsive phenotype, as recently postulated [73]. Anaphylactic episodes in patients with SM are more frequent than among the general population [74], and can be found at frequencies that range from 22 [23] to 49% [72]. According to our series, the percentage of anaphylaxis among ISM patients who have skin lesions (ISM-positive) is of 15.4%, while this percentage increases to 67% among cases lacking skin lesions (ISM-negative) [23]. Insect stings are among the most frequent triggers for anaphylaxis in SM. The association between HVA and mastocytosis has been widely documented [23,31,75]. Insect sting anaphylaxis has been reported to affect SM patients at similar frequencies (5 [23] to 19% [72]) and it has been reported that patients suffering from mastocytosis and hymenoptera venom allergy are at risk of severe insect sting-triggered anaphylaxis [34]. Among these SM patients, 44% presented as ISM-negative in one of these reports [23]. In addition, it has also been documented that 88% of patients with HVA and increased sBT levels present a clonal MC disorder [31].

Hymenoptera venom anaphylaxis is the presenting symptom in a significant percentage of MCAS. Accordingly, in a report on 83 MCAS patients [30], 51 presented *KIT* mutation, 29 of whom (57%) showed HVA as the presenting symptom. Overall, several reports on HVA suggest that the frequency of mastocytosis and other c-MCADs is higher in patients with HVA than in the general population [30,31,34]. TABLE 1 shows the majority of cases with MCAS reported in patients presenting with HVA. In addition, several studies have found a higher prevalence of HVA in mastocytosis than food or drug hypersensitivity [23,30,75]. Such association between clonal MC disorders and HVA deserves further investigations in larger series of cases, in order to elucidate the mechanism of activation of clonal MC in case that may have or may lack specific IgE antibodies against venoms.

■ Mastocytosis & clonal systemic mast cell activation syndromes as risk factors for hymenoptera venom anaphylaxis

Assessment of the risk for anaphylaxis still represents a daily practice challenge for physicians. Recently, researchers from the American Academy of Allergy, Asthma and Immunology (AAAAI) and the European Academy of

Allergy and Clinical Immunology (EAACI) held a Practical Allergy Meeting (PRACTALL) focusing on how to assess the risk for anaphylaxis. Part of the activities and conclusions of the meeting focused on improved methods for identification of individuals at risk of anaphylaxis by specific allergens such as insect venoms [76]. Assessment of individual risk for HVA after insect sting, as well as for systemic and severe adverse reactions in patients undergoing VIT, is one of the most relevant challenges in daily practice, particularly in patients who carry clonal MCs. The most relevant predictive factors are discussed in the following section.

Serum baseline tryptase

During anaphylactic reactions, there is a variable increase in plasma/serum β -tryptase levels; such increase is detectable after a few minutes, it peaks at approximately 1–2 h later and returns to normal values within 24 h [77]. The increase in total tryptase (primarily α -protryptase) in SM has been associated with the total MC burden [77,78].

Severe adverse reactions to HVA have been associated with increased baseline serum tryptase levels [14–16,18,19,26,31,72,74,79,80], and a significant log-linear association between sBT levels and the need for an emergency intervention during the build-up phase of vespid VIT has been also recently described [81]. Nevertheless, the presence of SM, particularly in cases lacking CM (or clonal MC) has not been ruled out in a routine basis in several of the aforementioned reports [16,19,26,31,72] in which, because of this, both SM and clonal MCAS have most probably been underdiagnosed. In line with this hypothesis, two recent reports show a clear association between increased sBT levels over 11.4 $\mu\text{g/l}$ and SM or clonal MCAS in 48 out of 51 (94%) and 30 out of 34 (88%) patients, respectively [30,31]. Taken together, these results suggest that the presence of clonal BM MCs is a highly relevant risk factor for both HVA and severe adverse reactions to VIT, while the increase in sBT by itself should be considered as a relevant surrogate marker for anaphylaxis in both circumstances. Nevertheless, cases lacking clonal MCs who show increased sBT levels should also be considered more prone to suffering from anaphylaxis or severe systemic reactions after hymenoptera sting or VIT as also supported by our results [30]. Taken together these results indicate that sBT is of great value in cases lacking clonal MCs, as a predictor for the severity of symptoms in both HVA and VIT reactions.

Table 1. Summary of the results reported in different studies in the literature regarding the most relevant clinical and biological diagnostic features of patients with mastocytosis and hymenoptera venom anaphylaxis.

Study (year)	Patients [†] (n)	SM/CM [‡]	Skin lesion [§] n (%)	c-MCAS	sBT >11.4 µg/l n (%)	sBT >20 µg/l n (%)	Ref.
Brouet (1979)	1	1/0	1 (100)	0	NR	NR	[13]
Müller <i>et al.</i> (1983)	3	1/2	3 (100)	0	NR	NR	[14]
Kors <i>et al.</i> (1993)	5	5/0	3 (60)	0	NR	NR	[20]
Oude Elberink <i>et al.</i> (1997)	2	2/0	2 (100)	0	NR	NR	[18]
Fricker <i>et al.</i> (1997)	10	3/7	10 (100)	0	5 (50)	4 (40)	[15]
Biedermann <i>et al.</i> (1999)	1	0/1	1 (100)	0	1 (100)	1 (100)	[17]
Ludolph-Hauser <i>et al.</i> (2001)	13	0/13	13 (100)	0	12 (92)	6 (43)	[16]
Haerberli <i>et al.</i> (2003)	3	0/3	3 (100)	0	3 (100)	NR	[19]
Dubois <i>et al.</i> (2004)	17	17/0	8 (47)	0	NR	NR	[21]
Rueff <i>et al.</i> (2006)	29	8/21	NR	0	29 (100)	NR	[34]
Sonneck <i>et al.</i> (2007)	5	4/0	0 (0)	1	4 (80)	3 (60)	[28]
Akin <i>et al.</i> (2007)	1	0/0	0 (0)	1	0 (0)	0 (0)	[29]
González De Olano <i>et al.</i> (2007)	36	35/1	20 (64)	0	32 (89)	30 (83)	[23]
Potier <i>et al.</i> (2009)	6	5/1	5 (83)	0	6 (100)	5 (83)	[24]
Bonadonna <i>et al.</i> (2008)	16	16	2 (12.5)	0	16 (100)	9 (56)	[25]
González De Olano <i>et al.</i> (2008)	21	21	5 (24)	0	21 (100)	20 (95)	[35]
Bonadonna <i>et al.</i> (2009)	30	21/0	4 (13)	9	30 (100)	18 (60)	[31]
Dugas-Breit <i>et al.</i> (2010)	56	24/32	NR	0	46 (82)	NR	[26]
Guenova <i>et al.</i> (2010)	4	3/1	NR	0	4 (100)	3 (75)	[27]
Álvarez-Twose <i>et al.</i> (2010)	29	27/0	0 (0)	2	26 (90)	18 (62)	[30]

Results reported as number of cases (percentage in brackets).

[†]Among all patients included in each study, only patients with hymenoptera venom anaphylaxis were selected.

[‡]Patients diagnosed with systemic mastocytosis/patients diagnosed with cutaneous mastocytosis. The bone marrow study was not always performed following established methods and criteria for morphology, histology and immunohistochemistry, flow cytometry and KIT mutational analyses.

[§]Patients presenting skin lesions.

c-MCAS: Clonal mast cell activation syndrome; CM: Cutaneous mastocytosis; NR: Not reported; sBT: Serum baseline tryptase; SM: Systemic mastocytosis.

Assessment of mast cell clonality

Mastocytosis has been reported as a risk factor for anaphylactic shock or near fatal anaphylaxis [82]; in a large series of cases (n = 55), severe reactions were detected in 81.8% of patients with mastocytosis (as assessed by the presence of CM and elevated sBT) versus 17.3% of cases without evidence of mastocytosis (patients without skin involvement) [34]. In our experience, the severity of HVA is also higher among SM than nonmastocytosis patients [23,30]. As reported by Rueff *et al.* these results indicate that, “it is beyond doubt that patients with mastocytosis and HVA are at a high risk for very severe or even fatal sting reactions” [34].

Currently, it is widely accepted that increased sBT levels over 20 µg/l are highly suggestive of SM [32,50,51]. Recent data suggest that a lower threshold of 11.4 µg/l (at least in patients with a previous history of systemic reactions to hymenoptera sting) could be more efficient in selecting patients to assess MC clonality in the BM [31,64,73], mainly among patients who lack CM. As previously mentioned, in these patients,

BM MC numbers are usually lower than in SM cases who have skin lesions, there are no detectable BM MC aggregates in approximately 30% of the patients, and serum tryptase levels are frequently below 20 µg/l in more than a third of cases [30]. As a consequence, diagnosis of SM in such cases remains a challenge and should be based on careful evaluation of the three remaining minor criteria [51]. In other words, the relative percentage of ISM-negative or clonal MCAS patients among cases presenting with HVA in the absence of skin involvement, directly depends on the sensitivity and specificity of the methodological approaches employed to investigate such additional minor diagnostic criteria [30,31,81]. Since such highly sophisticated techniques frequently cannot be performed routinely out of reference centers, the Spanish Network on Mastocytosis (REMA) has developed a scoring system to predict for the simultaneous detection of both aberrant CD25⁺ expression and *KIT* mutation based on other easily and widely available parameters (REMA score): patient gender, the nature of clinical symptoms during MC-mediator release

episodes and sBT levels (TABLE 2) [30]. A REMA score ≥ 2 is associated with the highest sensitivity and specificity to predict for both MC clonality [30] and SM, and this score also has a higher efficiency than sBT alone [ÁLVAREZ-TWOSE *ET AL.*, UNPUBLISHED DATA]. Based on these results, we strongly recommend considering this simple method to be applied in routine clinical practice for the screening of clonal MC disorders lacking CM, including both clonal MCAS and SM. Such scores would avoid unnecessary BM studies, improve the identification of SM cases lacking CM who have low sBT levels and could represent an indirect method to assess clonality and thus, the risk for HVA or severe systemic reactions associated to VIT.

Other risk factors for adverse reactions after venom immunotherapy

Apart from the aforementioned factors, additional variables have been also reported as risk factors for emergency intervention during VIT:

- Patients receiving honeybee immunotherapy instead of wasp VIT;
- Rush or ultrarush protocol for the build-up phase;
- Elderly age;
- Short time interval between sting and initiation of VIT;
- Therapy with angiotensin-converting enzyme inhibitors or β -blockers;
- Several concurrent conditions, such as acute infections, altered host-defense mechanisms or occupations (e.g., beekeeper) may also worsen the final success of VIT [81,83].

Table 2. Scoring model proposed by the Spanish Network on Mastocytosis to be applied in routine clinical practice as a screening tool for underlying systemic mastocytosis and clonal mast cell activation syndromes in patients with hymenoptera venom anaphylaxis.

Variable		Score
Gender	Male	+1
	Female	-1
Clinical symptoms	Absence of urticaria and AE	+1
	Urticaria and/or AE	-2
	Presyncope and/or syncope	+3
Tryptase [†]	<15 ng/ml	-1
	>25 ng/ml	+2

Score <2: low probability of clonal MCAD; score ≥ 2 : high probability of clonal MCAD.

Sensitivity: 0.92; positive predictive value: 0.89; specificity: 0.81; negative predictive value: 0.87.

[†]Baseline serum tryptase.

AE: Angioedema; MCAD: Mast cell activation disorder.

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■ Diagnosis of IgE-mediated hymenoptera venom anaphylaxis in mastocytosis & clonal mast cell activation syndromes

Following an anaphylactic episode, the patient should always be referred to an allergist in order to perform a detailed anamnesis and carry out a complete allergic work-up. Venom hypersensitivity may be mediated by immunologic mechanisms (IgE mediated or non-IgE mediated), but also by nonimmunological mechanisms. Diagnosis of hymenoptera venom allergy is based on the clinical history, skin tests and measurement of specific IgE antibodies. Skin prick and intradermal tests, as proposed by the EAACI and the AAAAI [84–86], are the methods of choice among skin tests and it has been demonstrated that they can be safely applied in mastocytosis patients [23]. They should be performed at least 2 weeks after the sting and in cases with negative results they should be carried out again at 1–2 months after, to avoid the refractoriness period. Determinations obtained through *in vitro* tests, such as serum allergen-specific IgE levels measured by means of a radio-allergosorbent test or an immunoassay capture test, are also informative; however, the sensitivity for specific IgE (sIgE) in patients with prior history of a systemic sting reaction is lower with such *in vitro* tests than with intracutaneous skin tests [84]. The most frequent type of sensitization to hymenoptera species in patients with mastocytosis or clonal MCAS has been reported to vespidae [19,23,31]. In addition, in two series of 163 and 74 adults with mastocytosis [23,72], the median serum total IgE levels was low: 27 and 12 kU/l, respectively. This median significantly increased in the first report [23] to 58 kU/l (range: 5–914 kU/l; $p < 0.005$) when compared with serum total IgE levels of the patients with allergy (39/163 [23.9%]) with regard to the remaining mastocytosis patients (124/163 [76.1%]). Low levels of serum IgE in patients with mastocytosis have been attributed to an increased absorption of circulating IgE by the increased amount of MCs [19,87].

The prevalence of negative skin tests and normal sIgE in patients with HVA who attend medical clinics ranges from <10 to >20%, in the normal population [88]. Noteworthy, neither SM nor clonal MCAS have been excluded in a routine basis in the reports cited previously. Negative tests can also be associated with longer time intervals since the last sting [89,90], but they do not mean the patient is cured [91] or not at risk of a new anaphylactic episode.

Currently, it is known that subjects with anaphylaxis after hymenoptera sting and negative allergy tests might have unrecognized mastocytosis [92] and should be carefully assessed for clonal MC disorders [28], especially when they have increased sBT levels [31]. In fact, the absence of venom sensitization has been frequently observed in SM with HVA [14,15,20–22,87,92,93]. In our series, including ISM-positive and ISM-negative cases, as well as clonal MCAS, the number of cases lacking specific IgE was of 15% (6/41) [30]. Among all patients referred up to date to the REMA with HVA episodes (n = 56), an IgE-mediated mechanism could not be demonstrated in ten cases; interestingly, all these ten patients fulfilled criteria for ISM-negativity.

Due to the relevance of reaching a final diagnosis of hymenoptera venom allergy, the number of diagnostic tools for allergy has significantly grown in recent years, and tools to assess the risk for anaphylaxis have also received more attention [76]. Despite some limitations [94], among the new strategies and tools, the basophil activation test (BAT) has emerged as a diagnostic technique recommended for the study of IgE-mediated diseases, including hymenoptera allergy. BAT is based on the evaluation of allergen-induced basophil activation as measured by an increased CD63 and/or CD203c expression on PB basophils [95] and it could be used as a surrogate marker for the presence of allergen-sIgE on basophils, to confirm the existence of sensitization in the absence of a positive sIgE result in serological tests [95], especially in cases where allergen sIgE levels are low due to a rather low total IgE value (<10 kU/l) [94]. BAT has also been shown to confirm sIgE sensitization in patients with clinical history of HVA in the absence of positive skin tests or positive allergen sIgE [96]; it may also provide information about the culprit insect leading to a better definition of the type of immunotherapy required [96,97]. In line with these results, another study on 55 mastocytosis patients has reported positive BAT and histamine release tests in three patients in whom venom sensitization could not be identified by other routine methods [98].

If after skin tests, serum determination of IgE or BAT had been carried out, no IgE-specific antibodies can be demonstrated, additional *in vitro* examinations should be carried out to identify the immunological mechanism involved (e.g., IgG, basophil histamine release test and leukotriene release test) [34,84,99–101].

Nevertheless, results of these tests should be taken with caution since there are not enough data regarding their utility in detecting immunologic sensitization of SM patients who have HVA.

Venom immunotherapy in mastocytosis & clonal mast cell activation syndromes

■ Principles of venom immunotherapy

Currently, little information exists about the mechanism by which VIT modifies or suppresses immune responses of both basophils and MCs. Although decreases in IgE antibody levels normally require years, decreased numbers of tissue MCs, eosinophils and their mediators are detected during the first hours after specific immunotherapy, particularly during increasing doses of allergens [102].

Treatment of hypersensitivity to hymenoptera venom with VIT is the only method that can modify the course of the disease by reducing the severity of systemic reactions and improving patients' quality of life [103]. After a hymenoptera sting, the allergic individual responds to venom by producing cytokines, which drive to the production of IgE. VIT seems to induce a state of tolerance in the T-cell subsets through a shift from a Th2 to Th1 immunological response. By contrast, B-cells are stimulated to initially produce venom-specific IgE and IgG, but after a few months the production of IgE antibodies is suppressed while that of IgG (specially IgG₄) is further stimulated. Thus, hymenoptera venom immunotherapy can be considered as the most effective form of immunotherapy. Two clinical trials performed in the 1970s in the USA and in Europe, showed efficacy of VIT against new stings in 98% of patients treated with venom versus placebo or a whole-body extract [3,4].

Several differences exist between VIT and other immunotherapy approaches [104] (e.g., compared with immunotherapy using inhalants). First, the initiation regimens are usually faster, whereby maintenance doses are reached in a few days or weeks, with a safety similar to traditional regimens; second, with VIT, the maintenance dose is equal for all patients (100 µg; lower doses have shown no efficacy), while doses for immunotherapy with inhalants are frequently adapted to patient's tolerance and reduced in case of adverse reactions. Third, maintenance doses with inhalants are administered in a fixed interval, whereas in hymenoptera VIT intervals between doses vary from every 4 weeks during the first year, to 8 or even 12 weeks in some patients.

■ Indications of venom immunotherapy in mastocytosis & clonal mast cell activation syndromes

International guidelines recommend VIT administration in patients who have experienced systemic reactions and in whom an IgE-induced mechanism has been demonstrated, since it is an efficient therapy to reduce the risk of systemic and local reactions in subsequent stings [7]. Despite the fact that it has been suggested that 'prophylactic' VIT might be indicated in SM patients even without evidence of IgE sensitization, due to the striking coincidence of mastocytosis and HVA [105,106], it is currently believed that VIT should be only recommended to patients with IgE-mediated anaphylaxis. Nevertheless, it is recognized that patients with mastocytosis have lower levels of total serum IgE versus the normal population [23,72] at the same time, cell diagnostic tests have identified an immune-mediated mechanism in cases where specific IgE evaluation showed negative results [96]. Thus, additional studies might be recommended in patients with SM in whom no specific IgE antibodies are detected, prior to a final decision on VIT being made.

Guidelines recommend that diagnostic tests to detect sensitization should not be carried out in the general population without a history of systemic reactions since VIT might not be indicated in other situations [84]; however, preliminary results suggest that administration of VIT in patients with large local reactions may also reduce morbidity [107]. Since mastocytosis patients are at high risk of HVA, the question about whether VIT should be prescribed in patients with SM and a previous history of large local reactions after hymenoptera sting still remains to be elucidated and further studies are needed to ascertain whether large local reactions in SM should undergo VIT.

It has also been proposed that patients with SM and hymenoptera venom allergy to either bees or wasps, should be treated with immunotherapy for both venoms even if sensitization to only one venom had been demonstrated [106]. International guidelines recommend VIT only to the culprit insect [7], but there is still no consensus regarding the potential benefit of a double VIT in SM patients.

■ VIT protocols

According to international guidelines [6] administration of VIT should be performed in a monitored setting with skilled personnel and available emergency treatment and equipment.

Furthermore, patients who have experienced a HVA should always have adrenaline auto-injectors available, even when they are already on maintenance doses of VIT or have been re-stung with only local reactions.

Different protocols have been designed for initiation of VIT, to reach the protective dose of 100 µg, minimizing side effects and improving patient's convenience [104]. Depending on the time required to achieve the maintenance dose, such protocols are classified as the following:

- Ultrarush regimens
- Rush
- Cluster
- Traditional regimens

Once the 100 µg dose is reached, maintenance doses are administered with a 1-month-interval during the first year, increasing to every 6 weeks during the second year and to every 8 weeks during the following years, for up to 5 years [7]. Interestingly, good results with a 12-week-interval period have also been reported [108].

Several types of VIT protocols for the build-up phase have been used in SM patients [15,19,25,33,35,82,109], and a tendency to a higher number of emergency interventions in those patients who underwent a rush or ultrarush versus a cluster or traditional protocol has been reported [81]. In a series of more than 50 SM patients treated with VIT by an experienced group with a cluster regimen involving weekly administration of increasing doses of 4.11, 5, 10, 20, 40, 70 and 100 µg, VIT administration did not require to be stopped in any patient in the build-up phase because of severe adverse reactions [BONADONNA P, PERS. COMM.]. This supports the notion that this schedule or a similar one might be used in HVA patients with mastocytosis.

When an adverse event occurs during the build-up phase, it is recommended to repeat the last dose and to use premedication [110]. As for patients who have no SM or clonal MCAS, the recommended maintenance dose in SM patients is of 100 µg [7]. In patients presenting adverse reactions during the build-up phase in whom a maintenance dose of 100 µg could not be reached, replacement of the conventional immunotherapy extract by an amine-free extract (Aquagen SQ, ALK Allergologisk Laboratorium A/S, Horsholm, Denmark) allowed the target 100 µg dose to be reached in some cases [35]. Despite sting challenges are not usually carried out, they

have been recommended to assess the efficacy of VIT and detect unprotected patients [34]. In patients receiving 100 µg as maintenance dose who develop severe adverse reactions after re-exposure, it is recommended to increase maintenance doses to 200 µg or more [25,34,106].

■ Premedication

Although VIT is an effective treatment, local or systemic reactions might occur in up to half of the patients, especially during the build-up phase [7]. In order to decrease the side effects observed during VIT administration, different premedication drugs and protocols have been proposed, which include antihistamines H_1 and H_2 , montelukast and corticosteroids.

Antihistamines H_1 have demonstrated efficacy to reduce local reactions and to prevent generalized urticaria [111], but they did not show an impact in more severe systemic reactions. Nonetheless, Müller *et al.* reported a lower number of systemic reactions with a re-sting after discontinuing VIT, in patients treated with antihistamines compared with patients receiving pretreatment with placebo, who experienced systemic reactions after hymenoptera sting (6/21) [112]. In another recent study, the same author found immunological changes in patients treated with antihistamines H_1 , and concluded that antihistamine H_1 could increase the efficacy of VIT [113]. Antihistamines H_2 have also been used as VIT premedication but no clear benefit has been reported [114]. Regarding montelukast, Wöhrl *et al.* compared the efficacy of pretreatment with 10 mg of montelukast versus 5 mg of loratadine in patients receiving VIT; they found that pretreatment with montelukast could also reduce the frequency of local reactions [115]. Finally, usage of corticosteroids in association with antihistamines is recommended as premedication to improve VIT tolerance in patients who have previously suffered from systemic reactions during or after VIT [116].

Some of the most relevant studies regarding VIT administration in SM, support the prescription of premedication prior to the administration of VIT, either in the build-up phase or during the maintenance phase [33,35,36,117]. When an adverse reaction during the build-up phase of VIT occurs, it is widely accepted that the patient should repeat the last tolerated dose and be premedicated with, at least, antihistamines H_1 [110]. Depending on the severity of the adverse reaction, additional drugs might be added on premedication.

■ Anti-IgE therapy

Treatment with anti-IgE may also strengthen the benefits of VIT. For patients presenting severe reactions after hymenoptera sting, who also show recurrent severe side effects during VIT, which enable them to reach standard maintenance doses, anti-IgE therapy can be of help and should be evaluated on an individual basis [117–121].

■ Safety & effectiveness

The rate of VIT efficacy ranges from 80 to 95% in the normal population [7] and VIT is also the preferred treatment in SM patients with IgE-mediated HVA. Side effects of VIT in the normal population are not more frequent than those occurring during immunotherapy with pollen, mould and mite allergen vaccines, with a rate of systemic reactions of approximately 12% [122]. However, only a few of such adverse systemic reactions are severe. In an attempt to limit the occurrence of systemic reactions, it is necessary to increase our knowledge about the potential risk factors.

Mastocytosis has been reported as a risk factor itself for side effects during administration of VIT [35]; despite this, its use has been recommended to treat life-threatening reactions in this group of patients at high-risk of HVA. The percentage of adverse reactions during administration of immunotherapy in SM ranges from 10 [19] to 85% [21]. A recent review of mastocytosis and venom allergy [109] gathered results on 117 patients who had received VIT, who showed a mean rate of side effects and of systemic side effects of 23.9 and 20.5% (7.6% requiring adrenaline), respectively.

Both the effectiveness and the safety of VIT in mastocytosis probably represent the most relevant factors in deciding whether or not VIT should be recommended in SM patients who suffer a HVA. Together with treatment failure reports [19,21] and fatalities after hymenoptera sting in patients who underwent VIT [18], there are many studies that report efficacy of VIT in SM [15,25,35] at rates that range from 15 [21] to 85% [25]. The review study carried out by Niedoszytko *et al.* [109] gathered 81 patients, with a rate of efficacy of 76.1% for patients with sting challenge and 66.7% for patients with field-sting. In another review study collecting 75 patients undergoing VIT [82], Bonadonna *et al.* also reported an efficacy of 67%. Similar data were obtained by the REMA, with an efficacy of 75% in 21 SM patients undergoing VIT. TABLE 3 summarizes data from different studies in the literature, which report VIT administration in patients with mastocytosis.

Table 3. Summary of the results reported in different studies in the literature on venom immunotherapy in patients with mastocytosis.

Study (year)	Patients [†] (n)	SR [‡] (%)	Protection [§] (%)	Fatalities [¶]	Ref.
Muller <i>et al.</i> (1983)	2	0 (0)	1/1 (100)	0	[14]
Kors <i>et al.</i> (1993)	3	2 (67)	NR	0	[20]
Engler <i>et al.</i> (1994)	1	0 (0)	1/1 (100)	0	[33]
Oude Elberink <i>et al.</i> (1997)	2	1 (50)	0 (0)	2 [#]	[18]
Fricker <i>et al.</i> (1997)	10	2 (20)	5/6 (83)	0	[15]
Biederman <i>et al.</i> (1999)	1	0 (0)	1/1 (100)	0	[17]
Dubois <i>et al.</i> (2004)	7	6 (86)	1/7 (14)	0	[21]
Rueff <i>et al.</i> (2006)	48	9 (20)	26/33 (78)	0	[34]
Bonadonna <i>et al.</i> (2008)	16	2 (12.5)	11/13 (85)	0	[25]
González-de-Olano <i>et al.</i> (2008)	21	6 (29)	9/12 (75)	0	[35]
Total	111	28 (25)	55/74 (74)	2/74 (3%)	

Results reported as number of cases (percentage in brackets).

[†]Among all patients included in each study only patients with mastocytosis were selected.

[‡]Patients presenting systemic reactions during administration of venom immunotherapy.

[§]Patients without systemic reactions after re-sting.

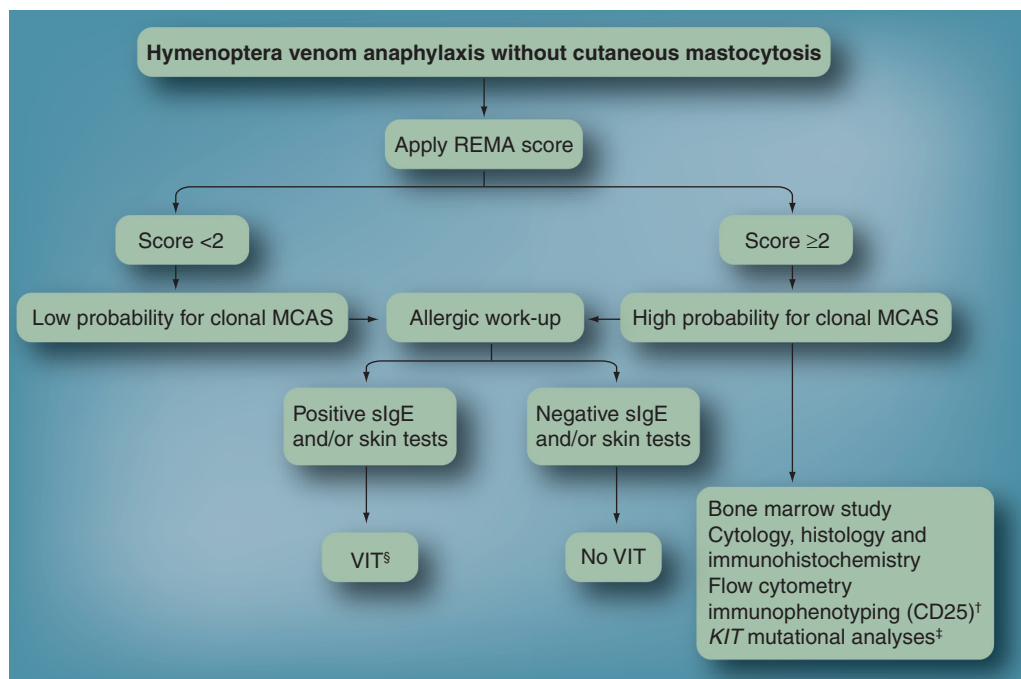
[¶]Fatalities after re-sting.

[#]Both patients received venom immunotherapy for 5 and 2 years, respectively, and venom immunotherapy was discontinued 9 years and 15 months prior to re-sting.

NR: Not reported; SR: Systemic reaction.

Although adverse reactions to VIT are more frequent among venom allergy subjects with (vs without) SM [7,100] and although fatalities following re-sting have been reported [21,105] even in patients who underwent immunotherapy

after they had stopped immunotherapy [18], VIT seems to be a safe and effective tool in SM patients with IgE-mediated hymenoptera venom allergy and should therefore be recommended in such cases.

**Figure 1. Proposed algorithm to be applied in routine clinical practice after a hymenoptera venom anaphylaxis episode in patients without cutaneous mastocytosis.**

[†]Specific approach for the detection of cells present at low frequencies should be used.

[‡]These studies should be performed in highly purified mast cells and in cases who have no *KIT* D816V mutation, other exon 17 *KIT* mutations should be investigated.

[§]See test for specific recommendations of VIT in ISM-negative and clonal MCAS.

MCAS: Mast cell activation syndrome; REMA: Spanish Network on Mastocytosis; sIgE: Specific IgE; VIT: Venom immunotherapy.

■ Venom immunotherapy duration

In the normal population, VIT is recommended to be administered for 3–5 years [123]. With such duration, VIT has been demonstrated to induce long-term protection in most patients, despite skin tests for serum sIgE levels possibly still remain positive [7]. When VIT is discontinued after a minimum 5 years of treatment, it has been shown to be associated with decreased venom IgE, negative skin tests and low risk for anaphylaxis [124]. Nevertheless, long-term or lifelong VIT has also been proposed in high-risk patients [7,19]. Thus, in patients with SM or clonal MCAS who usually have an increased (body) mast cell load and are assumed to have

a higher risk for HVA, administration of VIT might be extended over the standard duration or even lifelong [35,74,82,109].

Conclusion & future perspective

Patients suffering from systemic MC activation symptoms after a hymenoptera sting in the absence of mastocytosis-associated skin lesions, should be assessed by the REMA score. In patients with a score ≥ 2 , BM studies should be carried out since the presence of clonal BM MC appears to be a risk factor for HVA in subsequent stings and of systemic adverse reactions during VIT administration. BM studies should be performed in reference centers for MC-related

Executive summary

Venom immunotherapy in patients with mastocytosis & hymenoptera venom anaphylaxis

- Mastocytosis and other clonal mast cell activation syndromes:
 - In recent years, the presence of clonal mast cells (MCs) in a subset of patients with systemic symptoms attributed to MC activation who have no skin lesions has been reported and named as systemic MC activation syndromes (MCAS).
- Diagnosis:
 - In MCAS cases, bone marrow (BM) MC numbers are usually lower than in systemic mastocytosis (SM) with cutaneous mastocytosis, there are no detectable BM MC aggregates, and serum baseline tryptase (sBT) levels are often under 20 µg/l; thus, diagnosis of SM should be based on careful evaluation of the other three minor WHO criteria for SM and should be performed in reference centers using highly sensitive techniques for the immunophenotypic analysis and *KIT* mutational studies performed in fluorescence-activated cell sorter-purified BM MC to avoid false-negative results.
 - The Spanish Network on Mastocytosis has developed a scoring system based on a patient's gender, the clinical symptoms during anaphylaxis and sBT levels to predict for both MC clonality and SM.

Hymenoptera venom anaphylaxis in mastocytosis & clonal mast cell activation syndromes

- Prevalence:
 - Anaphylactic episodes in patients with SM can be found in frequencies that range from 22 to 49%.
 - Insect sting anaphylaxis has been reported to affect SM patients at frequencies that range from 5 to 19%, which confirms that patients with mastocytosis and hymenoptera venom allergy are at risk of severe sting anaphylaxis.
- Risk factors:
 - Severe adverse reactions to hymenoptera venom anaphylaxis have been strongly associated with increased baseline serum tryptase levels but the presence of SM in most of the reports has not been ruled out; therefore, both SM and clonal MCAS might be under diagnosed. Thus, clonal BM MC might be a direct risk factor for either HVA or severe reactions to venom immunotherapy (VIT), while the increase in sBT levels by itself should be considered as a potent surrogate marker for anaphylaxis.
 - Additional risk factors for VIT include honeybee immunotherapy instead of wasp, rush or ultrarush VIT protocol in the build-up phase, elderly age, short time interval between sting and initiation of venom immunotherapy and therapy with angiotensin-converting enzyme inhibitors or β -blockers.

Venom immunotherapy in mastocytosis & clonal mast cell activation syndromes

- Indications:
 - VIT is recommended in SM patients with IgE-mediated anaphylaxis.
- Protocols:
 - Depending on the time required to achieve the maintenance dose of 100 µg several VIT protocols have been proposed: ultrarush, rush, cluster or traditional regimens. A tendency to a higher frequency of adverse reactions in those SM patients who undergo a rush or ultrarush protocol, has been reported.
- Premedication:
 - When an adverse reaction during VIT occurs, the patient should repeat the last tolerated dose and be premedicated with at least antihistamines H_1 . Depending on the severity of the adverse reaction, additional drugs might also be used.
- Safety and effectiveness:
 - Regarding safety of VIT in SM, the mean frequency of side effects of VIT is estimated to be approximately 25% of cases; in turn, the rate of efficacy is of approximately 74% for patients after being re-stung.
- Duration:
 - Patients with SM or clonal MCAS have an increased risk for HVA, and should extend VIT over the standard duration (>5 years), or even be lifelong.

diseases, using highly sensitive flow cytometry immunophenotypic techniques specific for the detection of cells present at very low frequencies and *KIT* mutational analysis on fluorescence-activated cell sorter-purified BM MC. In MCAS patients with an IgE-mediated HVA, VIT is a safe and effective tool and it should be prescribed for periods that extend over the standard duration (>5 years), or even lifelong (FIGURE 1). Nevertheless, further multicentric studies are needed in order to establish the safety and efficacy of VIT in a larger series of SM patients and to determine whether different VIT protocols for the build-up phase or different VIT extracts might improve its tolerance and efficacy.

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