

Venom Immunotherapy: Risk Factors, Predictors, Duration, and Other Unmet Needs

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Abbreviation ACEI Angiotensin-converting enzyme inhibitor · ASM Aggressive systemic mastocytosis · BAT Basophil activation test · CCD Cross-reacting carbohydrate determinant · CMD Conventional maintenance dose · EMD Extended maintenance dose · HB Honey bee · HVA Hymenoptera venom allergy · ISM Indolent systemic mastocytosis · MMAS Monoclonal mast cell activation syndrome · PAF Platelet activating factor · PAF-AH Platelet activating factor acetylhydrolase · PLA2 Phospholipase A2 · sBT Serum baseline tryptase · SLIT Sublingual immunotherapy · SR Systemic reaction · VIT Venom immunotherapy · YJ Yellow jacket

Opinion Statement

Venom immunotherapy is the only preventative treatment modality for venom allergic individuals at this time. Given this, decreasing associated risks by improvement of its safety profile is paramount. This article will review new and recent advances in venom immunotherapy for the treatment of hymenoptera venom allergy. Advances include the identification of new risk factors for adverse reactions with immunotherapy, the expanded use of laboratory tests to identify those at highest risk of adverse reactions, the use of novel recombinant allergens for the diagnosis and treatment of venom allergic patients, and an update on known risk factors and predictors of immunotherapy failure. We also review the latest advances on emerging delivery mechanisms and alternate dosing schedules that aim to improve safety, tolerability, and cost of venom immunotherapy.

Introduction

Venom immunotherapy (VIT) has been around for almost a century, and in this time frame, significant advances have made this treatment modality a lifesaving procedure for individuals with venom hypersensitivity. The first reported use of VIT with whole body bee extract occurred in 1925 [1]. In 1956, Mary Loveless published the first report on the use of purified venom for testing and treatment of venom hypersensitivity [2]. Despite this, it would take an additional two decades for whole body immunotherapy to be proven ineffective in the first controlled trial of VIT [3]. VIT for hymenoptera venom allergy (HVA) has been shown to significantly reduce the risk of anaphylaxis to a flying insect sting to less than 5 % [4]. While advances in VIT have significantly improved its efficacy, risks continue to be present and as such require continued research into the development of emerging diagnostic and treatment options that can mitigate these risks. Recent advances in this area have focused on the identification of risk factors that

predispose individuals to complications from VIT. Mastocytosis is a significant risk factor for hymenoptera-induced anaphylaxis and is associated with increased severity of reactions. Laboratory predictors of adverse effects with VIT include tests such as serum tryptase, basophil activation test, and platelet activating factor. Improved diagnostic accuracy via the use of novel recombinant proteins may enhance the safety profile of VIT by decreasing the use of non-relevant venoms. New data indicates that previously considered risk factors for VIT such as antihypertensive medications (e.g., angiotensin-converting enzyme inhibitors and beta blockers) may in fact not be as significant as previously thought. Finally, new delivery mechanisms that may promote adherence and reduce the cost of VIT are also being investigated. The overarching goal with all these advances is to make the process of VIT as safe, practical, and efficacious as possible.

Treatment: new advances in identification of risk factors with VIT

Mastocytosis

Mastocytosis is a heterogeneous group of disorders characterized by aberrant mast cell proliferation. Clinical manifestation ranges from skin-only involvement as in urticaria pigmentosa to multi-organ involvement seen in systemic mastocytosis (SM). SM can be further classified as indolent systemic mastocytosis (ISM), systemic mastocytosis with a clonal hematologic disorder, aggressive systemic mastocytosis (ASM), and mast cell leukemia [5]. A diagnosis of monoclonal mast cell activation syndrome (MMAS) is considered in patients who have evidence of a clonal mast cell population but who fail to meet all criteria for SM.

Patients with mastocytosis are at increased risk of anaphylaxis to a variety of triggers including foods, drugs, and insect stings. In an otherwise healthy individual, anaphylaxis after an insect sting may be the first presenting symptom of mastocytosis. While HVA is estimated to occur in 3 % of adults in the general population, the prevalence of HVA is 10-fold higher in patients with any form of mastocytosis [6–8]. In fact, hymenoptera stings are one of the most common triggers for anaphylaxis in mastocytosis patients [6]. The exact prevalence of mastocytosis in HVA patients is unknown though previous studies have reported a range from 1 to 5 % [9–11•].

SM is also a risk factor for increased severity of systemic reactions (SRs). The risk from insect triggered reactions is highlighted by the fatalities reported in patients

with SM [12, 13]. Successful administration of VIT in patients with mastocytosis has previously been reported [14–16]. In one of the largest cohorts of SM patients undergoing VIT, there were four mild-moderate SRs during the induction phase in 84 patients [17••]. In a 2009 review of published data, Niedoszytko et al. noted systemic side effects in 20 % of SM patients undergoing VIT [7]. While initially controversial due to concerns of increased risk, VIT is now considered the standard treatment of choice for patients with HVA and SM [4, 18].

Although not completely elucidated, one of the presumed mechanisms for increased risks in SM patients is the increased mast cell burden that is present. Tryptase is a protease that is produced and stored in mast cell granules. Serum baseline tryptase (sBT) levels are an indirect marker of mast cell burden. Increased sBT levels have been associated with increased severity of reactions to HVA [19, 20]. In a large cohort of patients with HVA, 9.6 % had elevated sBT levels, and of these, 2.6 % were confirmed to have mastocytosis through further analysis [10]. Patients with a history of SRs to insect stings who fail to show evidence of venom IgE via skin testing or serology may have an underlying mast cell disorder and as such should be evaluated with a sBT level [4, 9, 11•, 16, 21]. Serum baseline tryptase levels are also expected to correlate directly with mast cell burden and therefore be predictive of anaphylaxis risk. However, evidence to the contrary was noted by van Anrooij et al. In their study of 329 patients with SM, the prevalence of venom induced anaphylaxis increased up to 47 % at a sBT level of 28 ng/mL. Further increases of sBT, however, were associated with decreased prevalence of venom-induced anaphylaxis [22].

While sBT levels >20 ng/mL are concerning for SM, it has been noted that some patients with SM may show only mild elevations in sBT. In a series of 379 patients with SRs to hymenoptera, patients with sBT levels >11.4 ng/mL were further evaluated for a clonal mast cell process via bone marrow biopsy, flow cytometric analysis, and KIT mutation analysis. Of these patients, 88 % were confirmed to have ISM or MMAS [11•]. In a recently described cohort of ISM patients without skin lesions, there was a marked male predominance; patients had lower sBT levels and were almost exclusively triggered by insect stings [23]. Findings such as these have raised the concern that using a sBT cutoff of >20 ng/mL may actually miss cases of SM in HVA patients. Furthermore, while a normal sBT level may provide reassurance, a subset of patients with normal tryptase levels (<11.4 ng/mL) and a history of severe hymenoptera reactions were also shown to have an underlying mast cell disorder [24••]. These patients were more likely to have higher sBT levels (median 8.6 ng/mL) and less likely to have cutaneous symptoms with previous anaphylactic episodes. A cutoff sBT of 7.95 ng/mL was associated with a sensitivity 69 % and a specificity of 83 %, while the absence of cutaneous symptoms had a sensitivity of 88 % and a specificity of 83 % in predicting an underlying mast cell process. Authors concluded that the absence of cutaneous symptoms during a severe anaphylactic episode due to HVA may be the most relevant factor in the identification of clonal mast cell disorders [24••].

Both conventional and accelerated protocols for induction of VIT have been used in patients with SM and HVA [14–16, 25]. In one study, SRs were seen in 5 % of patients and were more likely to occur in those undergoing a modified rush protocol that reached the maintenance dose (MD) in 7 days [17••]. A different study used a 3-day rush protocol and noted a SR in only one of nine patients [26]. An ultra-rush protocol that reached MD in 12 h was utilized in a patient with cutaneous mast cell disease without any systemic complications [27].

One approach to mitigate risks of adverse reactions is the use of omalizumab prophylactically and during VIT. In a patient with ISM and recurrent SRs with VIT, premedication with 300 mg omalizumab prevented further SRs until the omalizumab dose was weaned to 150 mg (patient experienced a SR with VIT after the third 150 mg dose) [28, 29]. A patient with MMAS, honeybee (HB) allergy, and a SR with VIT underwent pretreatment with three doses of omalizumab at 300 mg starting 10 weeks prior to VIT. He subsequently underwent a 3-day rush protocol requiring minor dosing modifications only. Monthly doses of omalizumab were continued for an additional 6 months with no further VIT-related adverse reactions during this period or after omalizumab was stopped [30].

Efficacy of VIT in patients with SM varies widely. Field sting induced SRs in patients with SM on VIT can occur in up to 85 %, whereas sting challenge-induced SRs occur in up to 40 % of patients depending on the study population [9–11•, 14–17••, 19, 25,]. Cumulatively, reactions on re-stings are estimated to occur in approximately 30 % of SM patients on VIT [7]. Consequently, despite VIT, these patients should continue to carry self-injectable epinephrine.

Length of VIT treatment in patients with mast cell disorders has not been addressed specifically though fatalities despite VIT would indicate that the consideration for lifelong VIT is a reasonable one [12]. While there are no specific studies on extended maintenance doses (EMDs) in SM patients, one study noted 86 % efficacy using an EMD schedule of 3 months after 3 years on a conventional maintenance dose (CMD) schedule [17••]. Similarly, there is no standard recommendation for increasing the MD in patients who have experienced SRs while on VIT, though in general, this practice has been considered protective [17••, 25]. In a small retrospective study of SM patients with HVA, investigators treated 17 patients with an increased MD of 200 mcg of HB or yellow jacket (YJ) venom. There were no SRs on sting challenge completed 6–18 months after the start of VIT. This is in contrast to the 31 % of control patients with SM and on standard 100 mcg MD of venom who experienced a SR on the sting challenge [31].

Serum baseline tryptase

Serum baseline tryptase levels are predictive of reaction severity in HVA independent from a history of SM. Elevated sBT levels (≥ 11.4 ng/mL) were noted in 5 % of patients with HVA and correlated significantly with severity of reactions [20]. Other studies have noted elevated sBT levels in up to 10 % of patients with HVA [10, 32•]. Rueff et al. noted that sBT levels are an independent prognostic variable for severity of field sting reactions in untreated patients. Remarkably, the odds ratio for severity of field sting reactions increases linearly starting at sBT levels of 5 ng/mL [33]. One study noted that during VIT, the influence of sBT on risk of reactions was most prominent in those receiving vespid VIT [32•].

Basophil activation test

Basophil activation via measurement of CD63 or CD203c expression has been studied for diagnostic and prognostic utility in patients with HVA. Basophil activation test (BAT) showed a sensitivity of 87 % and specificity of 100 % for the diagnosis of wasp venom allergy in a cohort of patients with ISM [34]. BAT was shown to be more sensitive than intradermal testing in cases with suspected HVA and inconclusive serologic and skin testing [35]. A separate study failed to show clinical utility of BAT in patients with SM, HVA, and negative serologic

and skin test evaluation [36]. One limitation for this study, however, was its small sample size as only seven patients were included in the study group.

Several studies have shown BAT as predictive of systemic side effects with VIT. High basophil sensitivity as measured by the percentage ratio of basophil response at 0.1 and 1 mcg/mL venom dilutions has been shown to correlate with increased side effects with VIT [37]. Zitnik et al. noted a higher 0.1/1 basophil response index in a group of children undergoing HB VIT who also experienced systemic side effects during build-up. Basophil sensitivity significantly declined after 6 months of VIT and remained low after 2–4 years of VIT [38]. Kucera et al. compared basophil responsiveness with results of sting challenges in a cohort of patients who had completed a mean of 4 years of VIT. A positive BAT was noted in 80 % of patients who reacted to a sting challenge. Conversely, 87.5 % of non-reactors had a negative BAT [39]. In a different study of patients who had also completed a mean of 4 years of VIT, increased basophil response was noted in patients who reacted to field stings compared to those who tolerated field stings. [40] Taken together, BAT may be a useful adjunct for the diagnosis of HVA, may provide prognostic information for adverse reactions with VIT, and may help identify patients at increased risk of relapse after VIT. A limitation of BAT use in clinical practice is that only specialized laboratories have validated protocols for routine use which may not be available across all clinical settings [41].

Platelet activating factor

Platelet activating factor (PAF) has also been studied as a marker for reaction severity. PAF is a potent mediator of inflammation with multiple biological functions [42]. Serum PAF levels were shown to directly correlate with the severity of clinical reactions in a group of patients with SRs from different etiologies [43]. The activity of PAF is tightly regulated as it is rapidly inactivated by PAF acetylhydrolase (PAF-AH). Decreased baseline PAF-AH levels are associated with increased severity of SRs in patients with HVA. [44] Baseline PAF-AH may provide prognostic value in determining who is at risk for immunotherapy-related SRs. Extracellular PAF-AH circulates in a complex and is also known as lipoprotein-associated phospholipase A₂ (Lp-PLA₂). While an FDA-approved and commercial test for Lp-PLA₂ exists, it is designed to detect elevation of Lp-PLA₂, a marker for vascular inflammation, and is associated with increased risk of cardiovascular disease.

Recombinant allergens

One approach to decrease the risk of SRs with VIT includes the use of alternate allergens. Fellrath et al. utilized long synthetic peptides (LSPs) with overlapping sequences of the major HB allergen phospholipase A₂ (PLA₂) and noted specific T-cell hyporesponsiveness in subjects undergoing treatment. Therapy was tolerated well overall aside from two patients who experienced mild delayed cutaneous reactions [45].

Safety of VIT may also be improved by enhancing the diagnostic accuracy for HVA and decreasing the inclusion of unnecessary allergens. Due to cross-reacting molecules found in venom extracts, it is not uncommon for multiple venoms to be positive during the evaluation of HVA. While some patients may in fact be truly sensitized to a variety of venoms, others may show multiple

positive results on skin testing or serology based on cross-reacting carbohydrate determinants (CCDs) or sequence homology of venom peptides. BAT has proven useful in cases of double positivity due to CCDs, though its lack of commercial availability limits its routine use [46].

Recombinant allergens offer an additional approach to differentiate between cross-reacting molecules and true sensitization. Specific IgE antibodies to several recombinant allergens are now available for testing (ImmunoCAP solid-phase assay, Phadia, Uppsala, Sweden) and include rApi m 1 (PLA2) and two vespula venom allergens rVes v 5 (antigen 5) and rVes v 1 (phospholipase A1). Recombinant Api m 1 sensitivity ranges from 57 to 79 % depending on the study population [47, 48]. Recombinant Ves v 5 is associated with a sensitivity of 85 to 90 % [48, 49]. Using both rVes v 5 and rVes v 1 resulted in a sensitivity of 92 % in subjects with established vespula allergy [49]. Diagnostic accuracy was further improved by spiking a standard venom extract with recombinant allergen which resulted in a sensitivity of 97 % in a group of YJ allergic individuals [50••]. Though limited by lack of commercial availability, the use of other recombinant HB or vespula allergens (e.g., rApi m 2, rVes v 3) has been shown to improve diagnostic accuracy though this has not been a consistent finding [51–53]. While recombinant allergens have thus far only been used for diagnostic purposes in humans, a mouse model of wasp allergy showed that VIT using rVes v 5 was as efficacious and had lower adverse effects compared to conventional venom extract [54].

Additional risk factors

Additional risk factors for SRs during the build-up phase of VIT include HB VIT, younger age, use of accelerated schedules, the presence of venom specific IgE, longer interval between insect sting and VIT, and the use of antihypertensive medicines [32•].

Cardiovascular disease and medicines

The presence of cardiovascular disease and the use of cardiovascular medications have been considered risk factors for SRs and severity of SRs in HVA patients. In an emergency room cohort of patients presenting with anaphylaxis from a variety of inciting factors, antihypertensive medication use in aggregate was associated with increased organ system involvement and higher risk for hospital admission [55]. The use of angiotensin-converting enzyme inhibitors (ACEI) has inconsistently been associated with increased severity of SRs after an index field sting [33, 56]. Neither ACEI nor beta-blocker use were associated with increased risks during build-up or maintenance phase VIT [57••, 58]. A retrospective study of patients on VIT failed to show an increased risk of SRs with concurrent ACEI use [59]. Despite this, Bernkopf et al. noted that ACEI use increased the risk of SRs during a 3-day rush VIT protocol [60••]. One reason for the lack of consistent findings on the use of antihypertensive medicines and VIT risks is that many of the studies include only a small number patients on these medicines.

Accelerated schedules

Accelerated build-up schedules have been associated with increased risks with VIT. Ultra-rush protocols were associated with an almost twofold increased risk for emergent intervention with VIT [32•]. Risk factors with accelerated schedules include female sex, use of ACEI, and HB VIT in a cohort of patients

undergoing a 3-day rush protocol [60••].

Limited data exists on the safety of accelerated schedules in children. A retrospective study using a 1-day ultra-rush protocol noted SRs in 16 % of children with a predominance of reactions in females [61]. In a different cohort of children who also underwent a 1-day ultra-rush protocol, SRs occurred in 3.7 % of children compared to 7.7 % of adults, though this difference did not reach statistical significance [62]. Reactions were mild to moderate in both of these study populations.

Dosing regimens

While the current standard MD for venom is 100 mcg, evidence exists that a lower MD of 50 mcg is also effective in children. After 3 years of HB VIT using a 50 mcg MD, 86 % of children tolerated field stings without SRs [63]. Similarly, there were no SRs with field stings in a group of children who underwent 5 years of VIT using a MD of 50 mcg [64]. Golden et al. reported 95 % protection to field stings in children who received VIT for a mean of 3.5 years using a MD of 100 mcg [65]. Efficacy is likely multifactorial with factors such as the amount of MD used, the type of venom, and the duration of VIT all playing a role.

After MD is reached, the interval between doses is increased to 4 weeks. Current practice parameter guidelines note the recommendation by field experts to increase the MD interval further after 12–18 months at 4-week intervals (6-week intervals for 12–18 months then 8-week intervals) [4]. Further support for the efficacy of EMD intervals comes from two recently published studies. Cavalluci et al. used a gradual approach for EMD whereby patients were extended to 3-month intervals after 3 years of VIT given every 5–8 weeks. During the EMD phase, 24 % of patients experienced local reactions only [66]. In a different study by Simioni et al., an EMD interval of 3 months was reached 4.5 months after VIT was started. After 5 years of VIT at the 3-month EMD interval, the interval was further extended to 4 months. Re-stings in the EMD group were tolerated without reaction 93.5 % of the time, whereas only 81.5 % of re-stings were tolerated in a control group on a CMD schedule [67••]. Reactions ranged from mild to moderate in both groups, though large local reactions (LLRs) were more frequently seen in control patients. Interestingly, there was no difference in reactions between those on an EMD interval of 3 months or 4 months [67••].

Predictors of VIT failure

Current practice guidelines in the US recommend a VIT course of 3–5 years except in cases considered to be high risk. These risk factors include severity of index sting, SRs while on VIT, HB allergy, underlying medical conditions, age, use of concomitant medicines, elevated sBT level, and the likelihood of future stings [4]. Consistent predictors for VIT failure as assessed by reactions following sting challenges or field stings include the presence of mastocytosis and the use of HB VIT [7, 68, 69••]. HB allergy is also associated with increased risks of VIT side effects [32•, 68, 69••]. While not completely understood, it is theorized that increased risks with HB VIT may be related to a difference in quality of extracts or insufficient dosing of standardized HB venom.

Serum baseline tryptase levels have not consistently been predictive of VIT failure. Haerberli et al. noted a significant association between sBT levels and VIT

failure in vespid allergic individuals as assessed via sting challenges [19]. Interestingly, a different study failed to show an association between elevated sBT level and risk of VIT failure though authors could not exclude smaller effects of sBT in their study population [68]. When adjusting for the presence of mastocytosis as a confounder, sBT levels less than 20 ng/mL did not result in increased VIT failure [69••]. Despite the lack of consistent association between elevated sBT and VIT failure, the risk of severe reactions with stings still remains in cases where sBT is elevated. As such, elevated sBT levels are an indication for prolonged VIT. Persistent basophil responsiveness as assessed with BAT has been associated with VIT failure as noted previously [39, 40]. ACEI use and its effect on VIT efficacy has been inconclusive as some studies note decreased effectiveness whereas others do not show a significant effect, though the latter may be due to the small number of study patients on an ACEI included [57••, 68, 69••].

Emerging therapies

Advances in the delivery of immunotherapy include the use of alternate delivery mechanisms. In a proof-of-concept study in 2011, 11 % of VIT doses given via an infusion pump were associated with local reactions only in three patients undergoing VIT build-up [70]. In a more recent study, the use of an infusion pump during build-up VIT resulted in local reactions with 32 % of doses. Only one of five patients in the study experienced a delayed reaction that consisted of angioedema [71]. In patients with LLRs to HB, sublingual immunotherapy (SLIT) using HB venom significantly decreased the peak diameter of local reactions with no adverse effects noted during induction or during the 6 months of maintenance therapy [72]. A different study by Patriarca et al. evaluated the safety and efficacy of SLIT using vesperula venom extract in 21 individuals with HVA. Two patients experienced mild reactions during the induction phase whereas there were no reactions during the maintenance phase. Of the four patients that had field stings, one patient experienced a LLR and one patient experienced throat constriction twice after field stings. Interestingly, there were no immunologic changes noted in the study group [73]. The small number of patients in this trial, however, makes it difficult to generalize the findings to a larger population.

Conclusions

Improved safety, delivery, and efficacy of VIT has been enhanced by recent advances in the diagnosis of HVA, identification of risk factors, and predictors of successful VIT. From these recent advances stem several recommendations in order to improve the safety profile of VIT. Allergists should strongly consider further evaluation for SM in venom allergic patients by obtaining a sBT level. Additional evaluation for SM should be contemplated in all patients with a history of life-threatening reactions to hymenoptera stings, patients whose insect triggered SRs are devoid of cutaneous symptoms and those who experience stinging insect reactions but have a negative diagnostic evaluation for HVA. Allergists should be aware that even minor elevations of sBT may still be consistent with a diagnosis of SM and as such further evaluation should be

considered. Current unmet needs include the lack of commercial availability of standardized recombinant allergens and laboratory tests to diagnose, treat, and monitor VIT. Large prospective studies need to be conducted to evaluate the safety and efficacy of recombinant allergens, EMD intervals, and the role of continued antihypertensive medicines in patients with HVA. Finally, the association between mastocytosis and HVA also warrants further investigation. While VIT has been shown to be helpful in patients with SM, optimal doses, dosing intervals, and length of treatment have not been clearly elucidated and require further research.

Compliance with Ethical Standards

Conflict of Interest

Dr. Karla E. Adams declares that she has no conflict of interest. Dr. Theodore M. Freeman declares that he has no conflict of interest.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

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