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ORIGINAL ARTICLE

Drug Allergy, Insect Sting Allergy, and Anaphylaxis

Venom immunotherapy in patients with clonal mast cell disorders: IgG4 correlates with protection

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Abstract

Background: Patients with clonal mast cell disorders (cMCD), systemic mastocytosis (SM) and monoclonal mast cell activation syndrome (MMAS), represent an increased risk for Hymenoptera venom anaphylaxis (HVA). Lifelong venom immunotherapy (VIT) is recommended; however, its efficacy and safety are controversial. Hence, we sought to evaluate the efficacy and safety of VIT in HVA patients with cMCD.

Methods: A retrospective study was conducted among 46 patients with *Vespula* venom allergy who had experienced severe HVA, 32 cMCD (22 with SM and 10 with MMAS) and 14 controls. There were no differences between cMCD patients and controls in age (58 vs 66) and duration of VIT (47 vs 48 months), respectively.

Results: During VIT, 11 (34%) cMCD patients experienced adverse reactions (ARs) (7% in controls), including 1 anaphylaxis. There were 23 re-stings in 17 (53%) patients during VIT. Of episodes, four (17%) presented with anaphylaxis, 14 (60%) presented with local reaction, and five (23%) were asymptomatic. In 11 episodes (48%), the patient did not take epinephrine, of these 8 (73%) presented with local reaction, and 3 (27%) were asymptomatic. Patient-based protection from anaphylaxis was 76% (4/17) in cMCD vs. 100% in controls during VIT. The venom-specific IgG4 concentrations increased during VIT ($P < .001$) although tryptase and IgE were unaltered.

Conclusion: Both safety and efficacy of VIT in cMCD patients were slightly reduced than controls. Severe ARs were rare. The elevated IgG4 levels may be a biomarker for efficacy of VIT in cMCD patients, as it correlates with protection from re-stings.

KEYWORDS

D816V mutation, hymenoptera venom anaphylaxis, IgG4, mastocytosis, venom immunotherapy

1 | INTRODUCTION

Clonal mast cell disorders (cMCD) comprise systemic mastocytosis (SM) and monoclonal mast cell activation syndrome (MMAS).[1,2] Common to these two conditions is the presence of mast cell (MC) clonality, as reflected in a mutation in codon 816 of *KIT* and/or occurrence of immunophenotypically aberrant MCs expressing CD25.[3] In patients with MMAS, the WHO criteria for SM are not fully met.[3]

Anaphylaxis is a well-known feature of cMCD; particularly, venom allergy represents an increased risk of severe, even fatal, sting anaphylaxis in these patients.[4,5] Although the overall prevalence of Hymenoptera venom-induced anaphylaxis (HVA) is approximately 25% in patients with SM,[6] the underlying reason(s) for this association remains elusive. The aggravated risk of severe HVA might be due to increased MC burden, perivascular aggregation of MCs and an amplified IgE reaction due to the presence of D816V *KIT* mutation.[7] These findings stress the importance of

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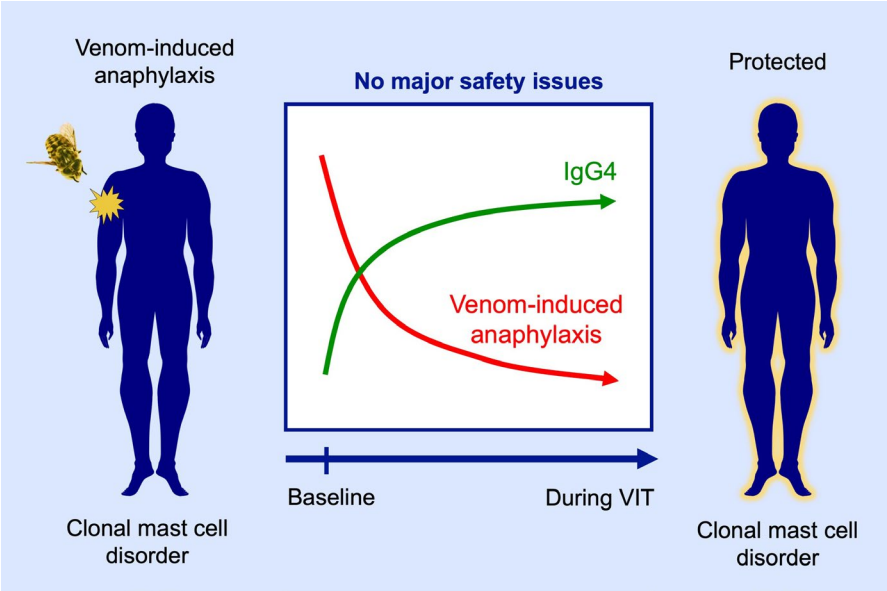

GRAPHICAL ABSTRACT

Patients with clonal mast cell disorders (cMCD) convey increased risk for venom-induced anaphylaxis and lifelong venom-immunotherapy (VIT) is recommended, when appropriate. VIT appears to be rather safe and effective in cMCD patients, as the risk of severe adverse reactions are rare. Elevated levels of venom-specific IgG4 correlates with protection from re-sting anaphylaxis in cMCD patients during VIT.

accurate diagnostics; therefore, underlying cMCD should be considered in patients with HVA that have elevated serum baseline tryptase levels (sBT) (≥11.4 ng/mL). Additionally, sensitization against Hymenoptera venom components should be confirmed by skin prick test and/or serum-specific IgE assays. However, it must be noted that patients with cMCD and HVA may lack sensitization to venoms.[4,8]

Venom immunotherapy (VIT) has been used for treatment of patients with diagnosed cMCD and HVA since 1990s; however, increased adverse reactions (ARs) and reduced efficacy have been main concerns in earlier studies.[9] As many as 6 in 7 patients with cMCD had reactions to field re-stings, despite ongoing VIT treatment.[9] This raised concerns and controversies about current recommendations regarding necessity and duration of VIT in patients with cMCD. Interestingly, more recent studies found VIT to be safe and effective in patients with cMCD but acknowledged a reduced efficacy and more frequent ARs during the administration of VIT compared with the general population.[10-13] At present, there is no evidence that VIT induces sustainable tolerance in patients with cMCD. Hence, the current recommendation is to proceed lifelong VIT in these patients.[14] Additionally, the known markers of successful VIT (IgG4, IL-10, regulatory T cells) have not been specifically studied in cMCD patients.

Thus, there is a continuing unmet need for further studies regarding VIT in patients with cMCD, as the available observations are based on limited number of reports. Here, we sought to determine the safety and efficacy of VIT by evaluating ARs during the administration of VIT and assessing the severity of field re-sting reactions. Furthermore, we also analysed the efficacy by monitoring certain biomarkers before and during ongoing treatment in patients with cMCD.

2 | METHODS

2.1 | Patients and clinical procedures

Between January 2006 and December 2018, 396 consecutive adult patients (≥18 years old) have been referred to the Mastocytosis Centre Karolinska due to clinically suspected cMCD including patients with mastocytosis in the skin, patients with severe anaphylaxis or patients with elevated baseline tryptase levels of unknown origin. The final diagnoses, for example, SM or MMAS, were obtained after a comprehensive medical evaluation and bone marrow investigation following WHO criteria.[3] Moreover, sBT levels (Thermo Fisher) were measured.

Anaphylactic reactions were diagnosed in accordance with NIH clinical criteria, when either reduced blood pressure or associated symptoms such as syncope/presyncope and/or respiratory compromise were present accompanied by the involvement of the skin-mucosal tissue and/or gastrointestinal symptoms.[15] In cases where assessments were difficult because of insufficient documentation, only patients who had syncope episodes after exposure to a likely trigger (eg insect sting) were assessed to have anaphylaxis. When available, serum tryptase levels during acute episodes were applied to confirm anaphylaxis. The diagnosis of HVA was based on clinical history, skin prick test and/or allergen-specific IgE.[16]

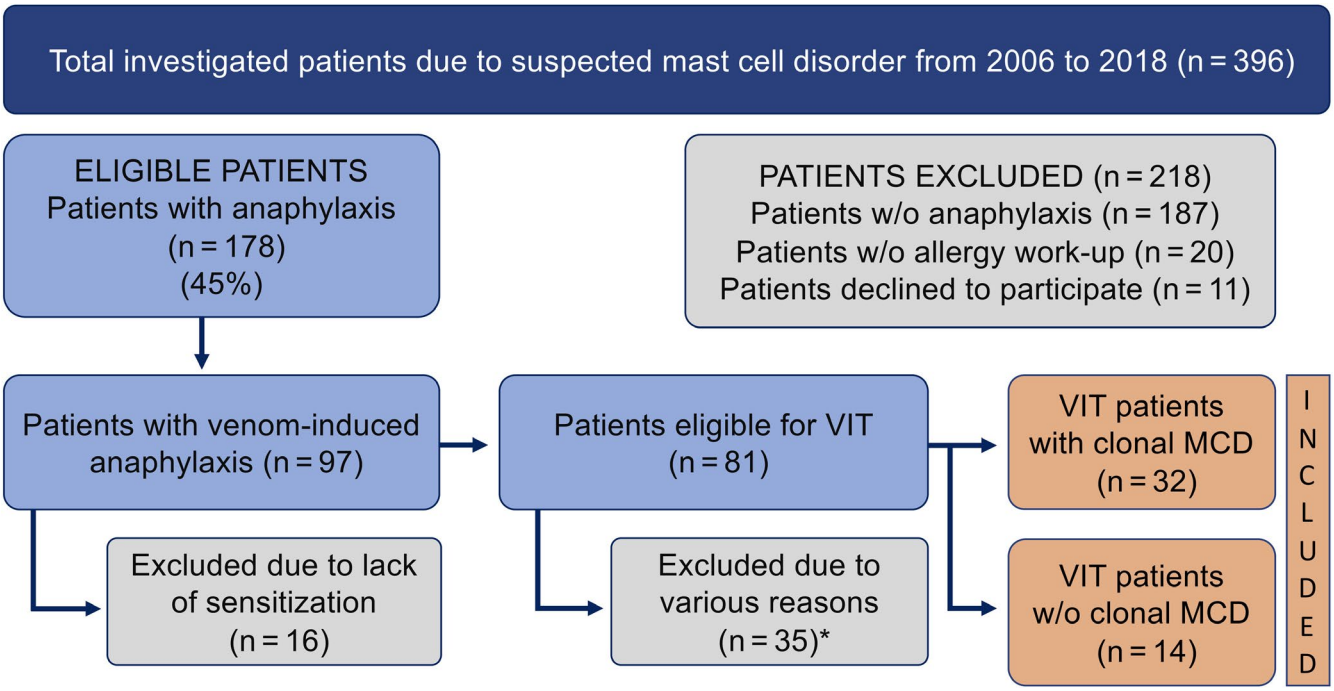

FIGURE 1 The flow chart illustrates the selection process of the study subjects. *35 patients were excluded due to various reasons (15 received VIT at other clinics, 7 had elevated baseline tryptase levels without underlying cMCD, 6 declined to undergo VIT, 4 had comorbidities with cancer, two patients were investigated during study start, and 1 patient was sensitized for honeybee only). Abbreviations: w/o, without; VIT, venom immunotherapy; MCD, mast cell disorders

2.2 | Allergy work-up

As previously described,[4] all patients went through a complete allergic work-up at Karolinska University Hospital Huddinge, allergy outpatient clinic including medical history, skin prick testing (SPT) with commercial extracts (ALK-Abelló A/S, Horsholm, Denmark) of standard aeroallergens, food allergens and allergen of Hymenoptera venom (honeybee and common wasp). The venom-specific IgE antibody test for honeybee and *Vespula* venom (ImmunoCAP Phadiatop®, Thermo Fisher, Uppsala, Sweden) was also performed and considered positive for values >0.10 kU/L. Moreover, serum concentrations of component-specific venom IgE (Ves r5, Ves r1, Api m1 and Api m10); venom-specific IgG4; and serum total IgE were determined by ImmunoCAP® (Thermo Fisher).

2.3 | Study design and subjects

A retrospective study was conducted. Data were collected through review of electronic patient records. Of 396 investigated patients, 178 had experienced at least one anaphylactic reaction (Figure 1). Among these, 97 patients with HVA were identified. After excluding 51 patients, 46 patients with *Vespula* venom allergy who fulfilled the criteria for VIT[14] were enrolled in this study (Figure 1). Of study subjects, 32 had diagnoses of cMCD and HVA. Additionally, 14 patients with HVA and normal sBT levels (<11.4 ng/mL) were included as controls to compare the safety and efficacy of VIT. The study was approved by Stockholm's Ethics Review Board (Dnr: 2011/1750/31/3 and Dnr: 2018/2621-31), and all enrolled patients provided their written informed consent to participate.

2.4 | Venom immunotherapy and follow-up

Venom immunotherapy was started with *Vespula* extract (ALK-Abelló) according to a 7-week traditional build-up schedule at the allergy outpatient clinic. Patients received incremental, weekly doses of depot venom extract subcutaneously until a maintenance dose (1 mL of 100 000 SQ-U/mL, corresponding to 100 µg) was reached. The achieved maintenance dose was then given every four (between May and October; high-risk season) or 6 weeks (between November and April; low-risk season for stings), and follow-up ended on 31 December 2018. All patients received premedication with HI blockers, 1-2 hours prior to VIT, and were observed 45 minutes after each injection. Extra drugs were given in case of acute reactions. Additionally, simultaneous treatment with omalizumab (Xolair®) during VIT was documented, when applied.

Blood samples were collected as part of routine patient care, and biological markers were in general analysed before VIT started and at different time points during VIT. Information about possible ARs was documented during the routine VIT visits, or as in few cases, patients reported late ARs by phone. Data about field re-stings during VIT and their outcomes were documented at the time of next follow-up visit and confirmed by the emergency room (ER) reports, when available.

2.5 | Statistical analysis

All analyses were performed using IBM SPSS Statistics 24.0 (IBM). Values of $P < 0.05$ were considered statistically significant. Frequencies were reported for categorical variables, and group

differences were analysed by using Fisher's exact test. Continuous variables were presented as median values and ranges. Because the distribution of the data was not normal according to Shapiro-Wilk test, the nonparametric Mann-Whitney *U* test or Kruskal-Wallis test was used to compare the group distributions, when appropriate. Additionally, when crude data analysis was significant, a post hoc analysis was performed using the Wilcoxon's matched pair rank sum test to detect alterations at different time points within groups. We used Spearman's rank correlation coefficient to demonstrate clinical relevance of venom-specific IgG4 during re-stings. Since re-stings were unpredictable, and it was impossible to assess the IgG4 on the day of re-sting, we used IgG4 concentrations closest to the day of re-sting occasions.

3 | RESULTS

3.1 | Patient characteristics and clinical data prior to venom immunotherapy

Of 32 patients with cMCD, 22 were diagnosed with indolent SM; 64% were males and had a median age of 61 (range 40-80) at diagnosis. Additionally, 45% of patients with SM (10/22) had cutaneous involvement, 36% had MC clusters in bone marrow biopsy, and 86% carried a *KIT* D816V mutation. They started VIT at a median age of 62 (40-81) years and received the treatment for a median period of 45 months (7-154). The remaining 10 cMCD patients were diagnosed with MMAS; 56% were males and had a median age of 55 (range 38-71). All MMAS patients had MCs expressing $CD25^+$ aberrant immunophenotype, whereas 22% concomitantly carried a *KIT* D816V mutation. They started VIT at a median age of 52 (38-66) years and received VIT for a median period of 77 months (9-104). Bone marrow examinations were offered to all but performed only in 4 of 14 controls, since most refused to undertake the procedure. Additionally, peripheral blood *KIT* D816V mutation was negative in all analysed controls (n = 11).

Subjects with cMCD were more often males (63%) than in the control group (43%), although this finding was not statistically significant (Table 1). Serum baseline tryptase levels were significantly higher in patients with cMCD, whereas total IgE were significantly higher in controls (Table 1). Moreover, controls presented with significantly higher levels of venom-specific IgE and component rVes v5 ($P = .001$; Table 1). To note, 57% of patients in control group suffered from cardiovascular comorbidities including hypertension and/or angina pectoris prior to the culprit sting reaction ($P < .001$; Table 1). Regarding the severity of culprit reactions, both cMCD patients and controls presented with severe HVA, where syncope frequently occurred (82% of the patients with SM, 56% with MMAS patients and 57% in controls). Additionally, controls frequently presented with skin symptoms ($P < .001$); otherwise, there were no significant differences between the two groups in terms of reaction severity (Figure 2).

VIT started in subjects with cMCD at an earlier age with a median of 58 years compared to 66 years in controls (Table 2). The duration of VIT was similar between the two groups and varied among individual patients due to the retrospective nature of the study. A total of seven patients discontinued VIT mainly due to the appearance of comorbidities (including cancer) or were referred to home clinics for practical reasons.

TABLE 1 Comparison of demographic, clinical and laboratory characteristics of cMCD patients with HVA compared to controls with HVA prior to VIT

Total, n = 46 Age ≥ 18 y	Clonal MCD (n = 32)	Controls (n = 14)	*P*-value
Male gender, n (%)	20/32 (63)	6/14 (43)	.333[a]
Age at diagnosis, median (range)	59 (38-80)	66 (46-78)	.129[b]
sBT levels (ng/mL), median (range)	18 (3.2-68) (7 NA)	5.3 (2.4-11)	**<.001**[b]
Total IgE (kU/L), median (range)	25 (2.6-1000) (11 NA)	77 (28-790) (2 NA)	**.033**[b]
Presence of atopy, n (%)	10/32 (31)	3/14 (21)	.724[a]
Positive SPT for wasp, n (%)	22/31 (71) (1 NA)	10/13 (77) (1 NA)	1.000[a]
Positive ImmunoCAP for wasp, n (%)	26/28 (93) (4 NA)	12/12 (100) (2 NA)	1.000[a]
Wasp-specific IgE (kU/L), median (range)	0.54 (0.09-48) (10 NA)	5.3 (0.12-50) (3 NA)	**.007**[b]
Component rVes v 5 (kU/L), median (range)	0.30 (0.1-25) (13 NA)	6.2 (0.11-60) (1 NA)	**.001**[b]
Component rVes v 1 (kU/L), median (range)	0.10 (0.1-75) (15 NA)	0.10 (0.1 −0.16) (11 NA)	ND
Wasp-specific IgG_4 (mg/L), median (range)	0.52 (0.04-8.9) (22 NA)	2.20 (13 NA)	ND
Syncope from wasp sting prior to VIT	24/32 (75)	8/14 (57)	.301[a]
Comorbidity with CVD prior to first sting, n (%)	2/32 (6)	8/14 (57)	**<.001**[a]

Abbreviations: cMCD, clonal mast cell disorders; CVD, cardiovascular disease; HVA, Hymenoptera venom anaphylaxis; NA, not analysed; ND, not done; sBT, serum baseline tryptase; SPT, skin prick test; VIT, venom immunotherapy.
[a]*P*-values were calculated using Fisher's exact test;
[b]*P*-values were calculated using a 2-tailed Mann-Whitney *U* test; bold indicates statistical significance ($P < .05$).

3.2 | Adverse reactions during VIT

Among patients with cMCD, 11 (34%) experienced ARs, eight (73%) during the induction phase and three (27%) during maintenance. The total number of episodes was 16, and epinephrine was administered twice (Table 2). Nine of these episodes were only local reactions, and six involved milder systemic reactions (without respiratory/cardiovascular symptoms). Conversely, ARs in controls were limited to one patient (7%) who reacted with milder systemic reaction (Table 2). Anaphylaxis was observed only in one patient with SM who received simultaneous immunotherapy against wasp and honeybee and occurred nine months after the maintenance phase of VIT had started. The patient presented with flush and general weakness a

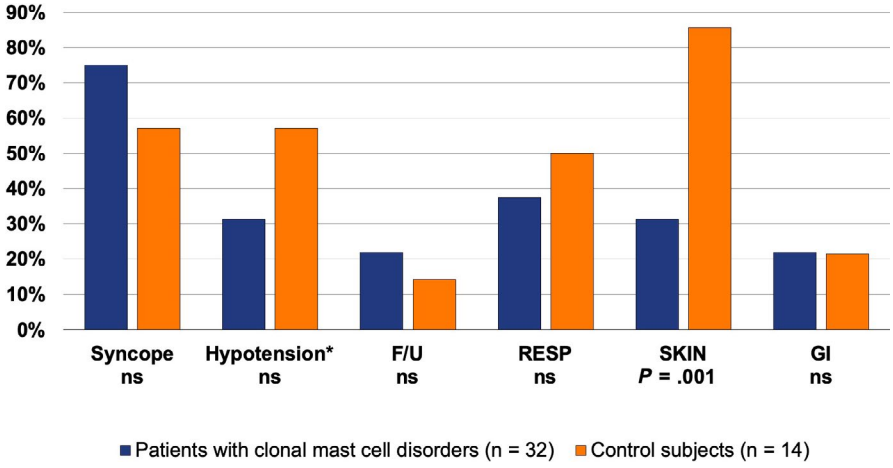

FIGURE 2 Clinical symptoms in patients with cMCD and controls during the culprit anaphylactic reactions to wasp sting prior to VIT. Statistical analysis performed by Fisher's exact test. *Hypotension, objectively verified. Abbreviations: F/U, faecal and/or urinary incontinence; GI, gastrointestinal cramps, nausea, vomiting and diarrhoea; RESP, respiratory symptoms; SKIN, local swelling and redness, itching

TABLE 2 Group comparison of adverse reactions during VIT

	Clonal MCD (n = 32)	Controls (n = 14)	*P*-value
Age at start of VIT, median (range)	58 (38-81)	66 (45-79)	.127[a]
Total VIT duration (months), median (range)	47 (7-154)	48 (13-65)	.277[a]
Patients with adverse reactions from VIT, n (%)	11/32 (34)	1/14 (7)	.073[b]
Total number of episodes with adverse reactions (n)	16	1	ND
Mild systemic adverse reactions, n (%)	7/16 (44)	1/6 (17)	ND
Patients with anaphylactic reaction from VIT, n (%)	1/32 (3)	0/6 (0)	ND
Total number of injections, n	1 781	493	ND
Number of injections needed per adverse reaction, n	111	1	ND
Number of injections needed per anaphylaxis, n	1 781	ND	ND
Use of adrenaline in adverse reaction, n (%)	2/16 (18)	0/1 (0)	ND

Abbreviations: cMCD, clonal mast cell disorders; ND, not done; VIT, venom immunotherapy.
[a]*P*-values were calculated using a two-tailed Mann-Whitney *U* test.
[b]*P*-value was calculated using Fisher's exact test.

few minutes after receiving both VIT injections. The blood pressure was initially normal (122/70 mm Hg). Despite receiving immediate treatment with epinephrine, 3-4 minutes later the patient had documented hypotension (80/44 mm Hg). He received another dose of epinephrine and intravenous fluid before he was transferred to the ER. Afterwards, VIT could be resumed, however, solely with wasp extract and in conjunction with omalizumab (Xolair@) treatment (300 mg q2weeks). The omalizumab treatment has been continued, and no further incidences occurred since then. Omalizumab protection was applied in two other patients (diagnosed with SM and MMAS, respectively), and the treatment was discontinued after 54 and 20 months, respectively. Thereby, VIT was tolerated, as these patients still receive VIT. We needed to apply omalizumab in these patients to achieve maintenance doses since it has been reported in the literature with successful results to allow administration of VIT.

3.3 | Protection from re-sting reactions while ongoing VIT

A total of 17 (53%) patients with cMCD were re-stung in 23 separate episodes. Epinephrine was used in 12 episodes. One sting occurred during the induction phase and the remaining during the maintenance phase. Six episodes were asymptomatic, 10 resulted in local reaction, and three resulted in milder systemic reactions. Four episodes (in four separate patients) were assessed to be anaphylactic reactions, and all four patients used intramuscular epinephrine and sought emergency care (Table 3). Table S1 shows the main characteristics of 5 patients with anaphylaxis during VIT (four anaphylaxis during re-stings and one anaphylaxis as AR). Interestingly, these five patients had positive SPT for wasp at baseline (Table S1), whereas only 22 patients in overall cohort (71%) (Table 1). Nevertheless, this was not clinically significant. Meanwhile, five (35%) controls were re-stung in a total of eight episodes, which occurred during the maintenance phase of VIT (Table 3). Epinephrine was used in two episodes, although no anaphylaxis was observed.

During VIT, clinical symptoms from re-stings were found to be less severe compared to culprit reactions. Most re-sting reactions in patients with cMCD were limited only to skin; moreover, 23% were asymptomatic. In contrast to the culprit reactions, where 75% of patients with cMCD had syncope, only one patient (5%) had syncope during re-stings ($P < .001$). Consequently, we observed 76% (13/17) patient-based and 83% (19/23) episode-based protection from anaphylaxis in patients with cMCD during field re-sting reactions ($P < .001$).

TABLE 3 Comparison of patients with cMCD and control subjects regarding re-sting reactions during VIT

	Clonal MCD (n = 32)	Controls (n = 14)	*P*-value
Patients who were re-stung during VIT, n (%)	17/32 (53)	5/14 (35)	.346
Number of patients with anaphylactic reaction, n (%)	4/17 (24)	0 (0)	ND
Protection from anaphylaxis per subject, n (%)	13/17 (76)	0/5 (100)	ND
Number of episodes of re-stings (n)	23	8	ND
Episodes with anaphylactic reaction to re-sting, n (%)	4/23 (17)	0/8 (0)	ND
Protection from anaphylaxis per episode, n (%)	19/23 (83)	8/8 (100)	ND
Use of adrenaline in episode of re-sting, n (%)	12/23 (52)	1/8 (13)	.095

Abbreviations: cMCD, clonal mast cell disorder; ND, not done; VIT, venom immunotherapy.
P-values were calculated using Fisher's exact test.

3.4 | Dynamics of biomarkers in patients with cMCD during VIT

Serum concentration of wasp-specific IgG4 increased significantly in patients with cMCD over the period of VIT ($P < .001$; Table S2). In contrast, repeated measures of other biomarkers including plasma levels of sBT, total IgE, wasp venom-specific IgE or venom component rVes v5 did not show significant dynamics during VIT compared to baseline levels. Calculation of specific ratios incorporating IgG4 did not provide any additional significance beyond IgG4 levels alone (Figure S2).

On group level, the median of wasp-specific IgG4 before VIT was 0.52 mg/L (range 0.04-8.9) in cMCD (Table S2). Two outliers were identified (2.4 and 8.9 mg/L, respectively) at baseline, and both previously completed a 5-year VIT course (Figure 3A). Afterwards, VIT was restarted when both patients were diagnosed with cMCD. We also evaluated wasp-specific IgG4 levels in six cMCD patients in relation to nine re-sting reactions and found an inverse correlation between IgG4 concentrations and reaction severity ($P < .01$; Figure 3B). Since IgG4 could not be obtained on the day of the re-stings, we analysed the closest IgG4 values in relation to day of reactions (median 4 months, range 0-10). Two patients who were asymptomatic in three episodes when re-stung had wasp-specific IgG4 levels between 21 and 25 mg/L. Conversely, the only patient who reacted in two episodes during the same summer with mild systemic reactions presented with lower wasp-specific IgG4 levels, 10 and 11 mg/L prior to and after the stings. The remaining five patients who only experienced local reactions (<10 cm in diameter) had IgG4 concentrations between 8 and 21 mg/L.

4 | DISCUSSION

Our results support that VIT appears to be rather safe and effective in patients with cMCD and HVA as the risk of severe systemic ARs was rare. Additionally, significantly increased serum concentrations of wasp-specific IgG4 were detected; this is, to our knowledge, the first report demonstrating this phenomenon in cMCD patients.

While allergic reactions to foods and drugs have been reported in cMCD, the primary trigger for IgE-mediated severe, even fatal, anaphylactic reactions remains Hymenoptera stings.[17,18] In general, VIT induces protection from severe sting reactions in HVA patients during and after discontinuation of therapy.[19-22] Nevertheless, there have been controversies regarding its safety and efficacy in patients with cMCD. ARs to VIT have been reported in 29% of patients with cMCD compared to 14% in general HVA population.[12,23] VIT protocols applied during induction phase are also essential since increased frequencies of ARs were reported in rush- or ultrarush protocols.[24] Interestingly, no ARs were observed in a recent study of eight mastocytosis patients receiving VIT by ultrarush protocol.[25] Our study supports previous findings as we found a fivefold increased risk for ARs in cMCD patients compared to controls (34% vs 7%, respectively). Additionally, the ARs occurred mainly during the build-up phase, of which 44% were milder systemic reactions. Only one patient suffered from an anaphylactic reaction (3%). Of note, this patient was treated with both honeybee and wasp venom simultaneously, a procedure reported to increase the risk of ARs.[12] No patients in the current study had to be discontinued; however, VIT in patients with cMCD is not risk-free.

Efficacy of VIT is typically evaluated by sting challenges and reports from field re-stings; however, sting challenges are not performed in all clinics.[14] Several studies have reported on efficacy as the rate of protection from systemic reactions; nevertheless, no universally accepted grading system exists to classify the severity of systemic reactions.[26] This complicates comparison of the efficacy of VIT across different studies.[27] In patients with mastocytosis, the protection rate varied from 14% to 85% in a review of 10 studies with 201 patients.[12] Our results support VIT being an efficient treatment to prevent anaphylaxis, since only four episodes (17%) from field re-stings were classified as anaphylaxis. That implies a protection rate of 83%, in line with a previous report (86%).[10] Only one patient with cMCD reacted with syncope when re-stung compared to 75% during culprit reactions ($P < .001$). There were no fatalities in our series. However, since this protection can only be sustained during VIT, the current guidelines recommend lifelong VIT in patients with cMCD.

Notably, the severity of culprit reactions in patients with cMCD appears to be independent from concomitant cardiovascular diseases (CVDs) and use of beta blockers and/or ACE inhibitors since the control subjects more frequently presented with CVDs ($P < .001$) without experiencing more severe reactions. Thus, the

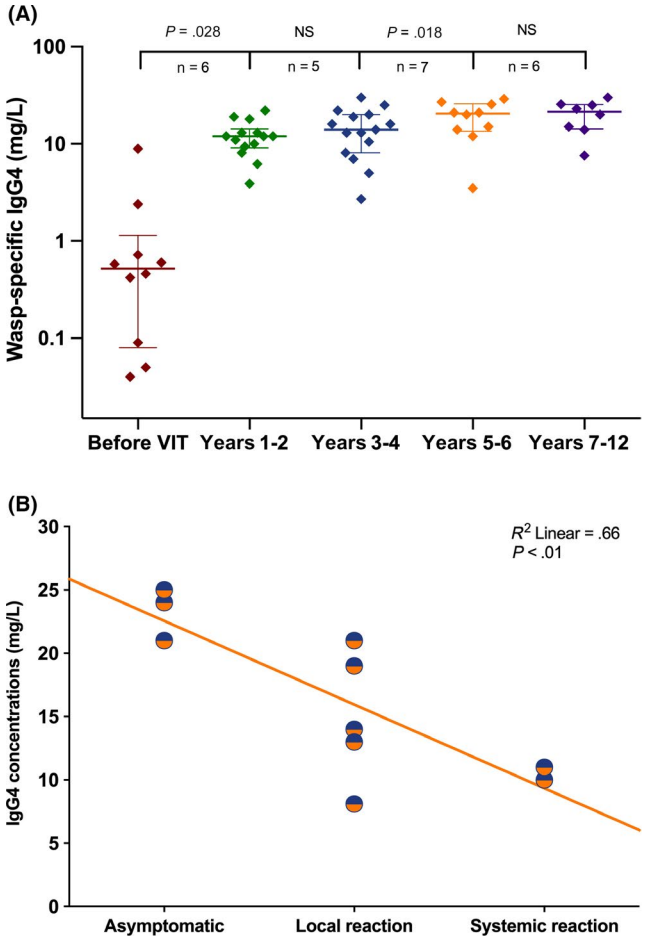

FIGURE 3 A, Dynamic of wasp-specific IgG4 levels in cMCD patients during VIT. Two outliers were identified (2.4 and 8.9 mg/L, respectively) at baseline, and both patients previously received VIT. *P*-values were analysed with Wilcoxon's matched pair rank sum test. A significant increase was noted between baseline and years 1-2 ($P = .028$) and between years 3-4 and years 5-6 ($P = .018$). n; number of matched patients at different time points. Different colours represent different time periods. B, Correlation between wasp-specific IgG4 levels and severity of re-sting reaction. The reactions were classified as asymptomatic, local reactions (<10 cm in diameter) and mild systemic reactions (without respiratory or circulatory compromise). Correlation was quantified by using Spearman's rank correlation coefficient

mechanisms leading to severe HVA in cMCD patients may be different from those of controls; for instance, they might be due to the inherent mast cell hyperreactivity. Additionally, the serum concentration of the venom-specific IgE and component-specific venom IgE rVes v5 (which is the dominating allergic epitope in Sweden) levels at baseline was lower in patients with cMCD compared to controls ($P = .013$). It is known that patients with SM typically exhibit lower levels of total and specific IgE, presumably due to the adsorption of specific IgE by the expanded MC burden[28]; however, whether this is true also for rVes v5 has not been previously investigated.

The immunological mechanisms underlying VIT efficacy have not been fully elucidated, although induction of peripheral tolerance and the generation of allergen-specific regulatory T (Treg) and B (Breg) cells appear to be cardinal features. Treg cells are characterized by IL-10 secretion that directly or indirectly suppresses effector cells including mast cells, and also have influence on B cells, suppressing IgE production and inducing the production of blocking-type IgG4 antibodies against venom allergens.[29-33] The earlier studies concerned patients from the general HVA population and acknowledged that specific IgG levels increased significantly during VIT but also decreased significantly when VIT was discontinued.[21,34-36] Since a protective effect was still evident even after VIT was discontinued, they concluded other immunological mechanisms rather than specific IgG were likely responsible. Nevertheless, these studies did not involve the IgG subclass, IgG4. Interestingly, another investigation reported that specific honeybee IgG4 concentrations remained increased two years after discontinuing VIT against honeybee, suggesting a long-lasting protection of specific IgG4.[37] This issue has been re-evaluated in a recent study in patients with HVA and demonstrated increased levels of wasp-specific IgG4 during VIT course, but concentrations declined substantially at 3- and 8-year follow-up after discontinuation of VIT.[38] Additionally, Golden et al[21] found that 88% of patients who reacted systemically to a sting during VIT had venom-specific IgG antibody levels ≤3 mg/L, thereby recommended that monitoring sIgG levels during VIT might be predicting residual risk of systemic reactions after a sting. Similarly, a later study reported that monitoring VIT efficacy was only possible in vespid-venom allergy, and the authors proposed that sIgG4 threshold for rVes v5 had the highest sensitivity to confirm tolerance.[39]

Currently, no studies have demonstrated sustained tolerance development in patients with cMCD. Because severe or fatal anaphylactic reactions with re-stings occur only in patients with cMCD after discontinuation of VIT, the immunological mechanism behind clinical efficacy may differ from nonclonal population.[12,14] However, the dynamic of IgG4 during VIT has never been analysed in patients with cMCD. We demonstrated that venom-specific IgG4 levels constantly increased during VIT and reached a 20-fold increase during the first 2 years of treatment (Figure 3A). Additionally, when we observed wasp-specific IgG4 in relation to re-sting reactions, we found an inverse correlation between IgG4 levels and reaction severity suggesting that IgG4 concentrations might reflect the clinical efficacy (Figure 3B). Furthermore, we also identified two patients who had anaphylaxis when re-stung, 4 and 11 years after having discontinued VIT. Both patients were later diagnosed with cMCD. The serum concentrations of wasp-specific IgG4 were clearly reduced in both (8.9 and 0.72 mg/L, respectively) before VIT was restarted compared to patients with cMCD who had been continued VIT (median IgG4 concentration 20.5 mg/L at years 5-6). Hence, treatment failure may be related to inadequate levels of IgG4. VIT could be administered more frequently in these patients to attempt to raise the serum concentration of venom-specific IgG4, thereby increasing efficacy.

Conversely, no significant alterations during different time points of VIT were observed regarding the levels of tryptase, total IgE, wasp venom-specific IgE and component-specific venom IgE. Notably, previous studies demonstrated both decreased and unchanged levels of venom-specific IgE during VIT.[23,40,41] In the study by Gonzales et al[23] with SM patients and HVA, venom-specific IgE

levels decreased for the entire group during VIT. However, only 6 of 21 patients had *Vespula* venom allergy.[23] Remarkably, their patients had relatively high median levels of specific IgE before VIT compared to our patients (4.15 KU/L vs 0.54kU/L). These might be contributing to the contradictory results. Meanwhile, tryptase levels remained unchanged during VIT in both studies.[23]

The main strength of this single-centre study was the homogeneity of the subjects enrolled, since all patients received VIT against wasp, with extract from the same manufacturer and followed by similar VIT protocols. We could therefore compare patients with cMCD to control patients in the same clinical settings. By contrast, paucity of study subjects and retrospective nature of the study lacking relevant data from all patients at all time points analysed were our limitations making difficult to generalize results. When reactions to re-stings were assessed, patient-reported data were unavoidably used and could include a recall bias. Use of epinephrine is a confounding factor that could not be avoided. Finally, several symptoms from systemic allergic reactions and panic attacks overlap, for example anxiety and tachycardia, and all these factors complicate clinicians' assessment of reactions.

In conclusion, our results suggest that both efficacy and safety of VIT are somewhat less reliable in cMCD patients, but the overwhelming benefit justifies the relatively small increase in risk, as severe ARs are rare. The patient-based and episode-based protective rate from field re-sting anaphylaxis were 76% and 83%, respectively; nevertheless, the efficacy of treatment was only measured during VIT course. Therefore, we support the notion that patients with cMCD should continue VIT indefinitely. Over the course of VIT, venom-specific IgG4 antibodies were increased significantly in patients with cMCD and an inverse correlation between IgG4 levels and reaction severity to field re-stings appears to exist. Using wasp-specific IgG4 to monitor clinical efficacy may allow us to schedule more individualized administration of VIT; however, this issue needs to be further explored.

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CONFLICT OF INTEREST

T. Gülen has received lecture fees from Thermo Fisher. C. Akin has received consultancy fees from Blueprint Medicines and Novartis and has a patent for LAD2 cells. J. Jarkvist and C. Salehi declare no relevant conflicts of interest.

AUTHOR CONTRIBUTIONS

JJ took active part in the acquisition, analysis and interpretation of the data, and drafting and revising of the manuscript. CS took active part in the acquisition and analysis of the data. CA analysed and interpreted the data and revised the manuscript critically. TG conceptualized and designed the study; collected, analysed and interpreted the data; and wrote and revised the manuscript. All authors approved the final submitted manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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5 **Appendix S1**

6 **SUPPLEMENTARY MATERIAL**

7 Table S1: Clinical and laboratory characteristics of anaphylactic reactions in patients with
8 cMCD during VIT

Study id	#2	#6	#16	#17	#22
Age at diagnosis	50	64	66	59	38
Sex (female = 0; male = 1)	1	1	0	1	1
Diagnosis	ISMs-	ISMs-	ISMs+	ISMs+	MMAS
Reaction pattern at baseline	Sync, SKIN, GI	Sync, RESP, SKIN	V, SKIN, GI	Sync, RESP	Sync, RESP, F/U
sBT (mg/L) before VIT start	42	17	29	25	4,3
Total-IgE (kU/L) before VIT start	67	44	190	16	12
SPT wasp, positive (Y/N)	Y	Y	Y	Y	Y
S-IgE / rVes v5 (kU/L)	1.8 / 3.8	0.78 / Na	0.34 / 0.37	<0.35 / Na	0.88 / Na
Overall VIT duration (months)	93	94	14	109	104
Anaphylaxis as AR and reaction pattern	Ax (BP, V, SKIN)	n/a	n/a	n/a	n/a
Duration of VIT at ARs (months)	12 (M)	n/a	n/a	n/a	n/a
Use of adrenaline during ARs	2 x	n/a	n/a	n/a	n/a
Anaphylaxis during re-stings and reaction pattern	n/a	Ax Sync, BP, RESP	Ax V, Resp	Ax BP, V, SKIN	Ax BP, Resp, GI
Duration of VIT at re-sting (months)	n/a	4 (M)	10 (M)	57 (M)	3 (I)
Use of adrenaline during re-sting	n/a	2 x	2 x	2 x	2 x

Abbreviations: VIT = venom immunotherapy; SPT = Skin prick test; sBT = serum baseline tryptase; M, maintenance; I, induction; Sync, syncope; V, vertigo; BP, hypotension; GI, gastrointestinal cramps, nausea, vomiting, diarrhoea; RESP, respiratory symptoms; Skin, local swelling, redness, itching; F/U, faecal and/or urinary incontinence

Table S2: Dynamics of biological markers during venom immunotherapy in patients with cMCD.

Clonal MCD (n = 32)	Before VIT	Year 1-2	Year 3-4	Year 5-6	Year 7-12	p-value
Wasp-specific IgG_4 (mg/L), median (range)	0.52 (0.04 – 8.90) (n = 10)	12.0 (3.90 – 22.0) (n = 14)	14.0 (2.70 – 30.0) (n = 15)	20.5 (3.50 – 29.0) (n = 10)	21.5 (7.60 – 30.0) (n = 8)	**<0.001**
S-tryptase levels (ng/mL), median (range)	18 (3.20 – 68.0) (n = 25)	16.5 (3.20 – 72-0) (n = 20)	16.3 (3.25 – 29.0) (n = 18)	15.0 (6.90 – 28.0) (n = 14)	15.0 (4.5 – 33.0) (n = 9)	0.712
Total IgE (kU/L), median (range)	25 (2.6 – 1000.0) (n = 21)	31 (4.9 – 260.0) (n = 19)	19 (2.5 – 980.0) (n = 16)	89 (5.6 – 440.0) (n = 13)	61.0 (5.5 – 260) (n = 9)	0.685
Wasp-specific IgE (kU/L), median (range)	0.54 (0.09 – 48.0) (n = 22)	0.95 (0.24 – 11.1) (n = 18)	0.82 (0.13 – 59.0) (n = 15)	2.30 (0.10 – 30.0) (n = 11)	1.25 (0.14 – 28.0) (n = 9)	0.253
Component rVes v5 (kU/L), median (range)	0.3 (0.10 – 25.0) (n = 19)	0.84 (0.10 – 6.20) (n = 17)	0.56 (0.10 – 33.0) (n = 16)	3.0 (0.10 – 22.3) (n = 11)	0.76 (0.09 – 14.0) (n = 9)	0.241

Abbreviations: cMCD; clonal mast cell disorder; VIT = venom immunotherapy; ND = not done. P-values between different time points were calculated using a Kruskal-Wallis test. n; number of tested patients at different time-points. Bold indicates statistical significance ($P < 0.05$).

Figure S1: Different ratios of wasp-specific IgG4 in cMCD patients during the VIT. A- The ratio of wasp-specific IgG4 to tryptase; B- The ratio of wasp-specific IgG4 to total IgE; C- The ratio of wasp-specific IgG4 to wasp-specific IgE; D- The ratio of wasp-specific IgG4 to wasp-specific IgE component rVes r5.

P-values were analysed with Wilcoxon's matched pair rank sum test at different time-points and * denotes a significant increase. All ratios were calculated in units of nanogram per millilitre. IgG_4 levels were converted from milligrams of antibody per liter to nanograms per milliliter and the variables total IgE, specific IgE and rVes5 were converted from kilounits of antibody per liter to nanograms per milliliter using the formula ($IgG_4 \times 1000/IgE \times 2.4$). Abbreviation: cCMD: clonal mast cell disorders.

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60 Fig. S1A

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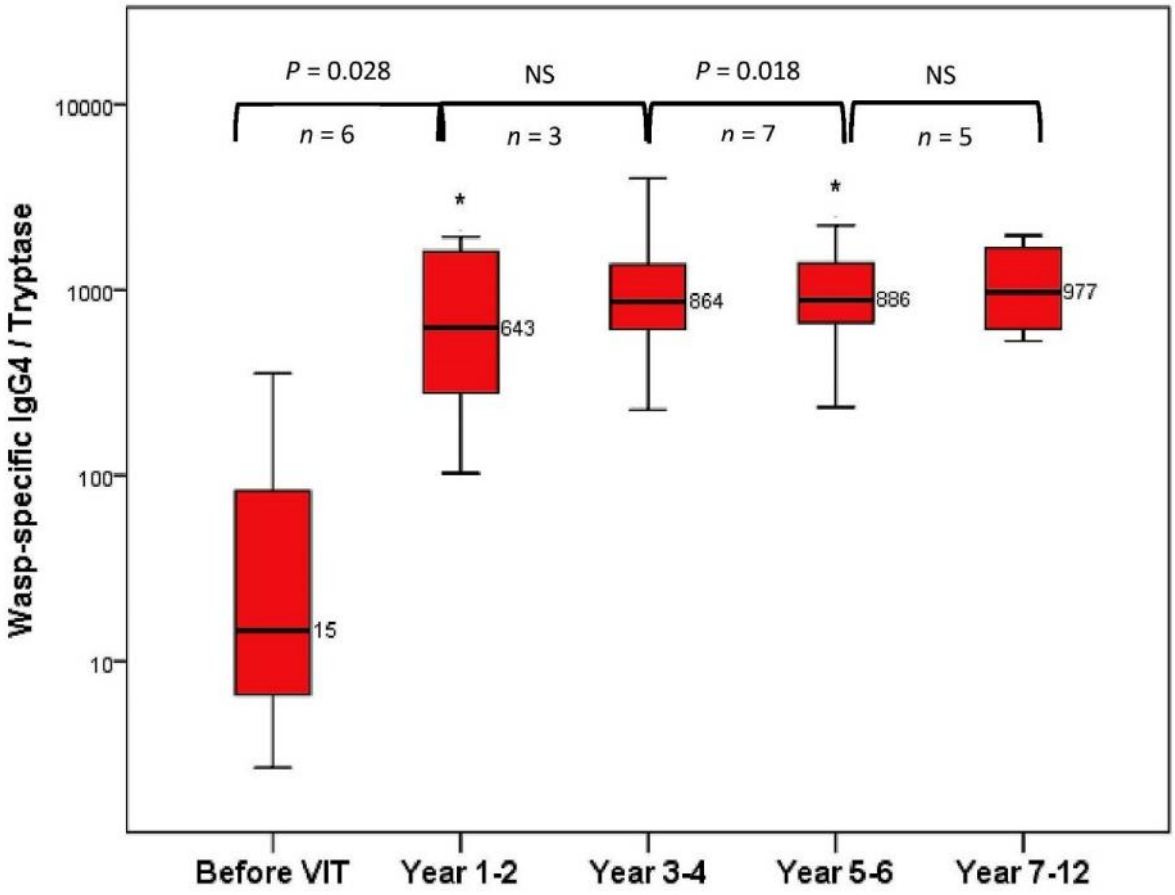

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Fig. S1B

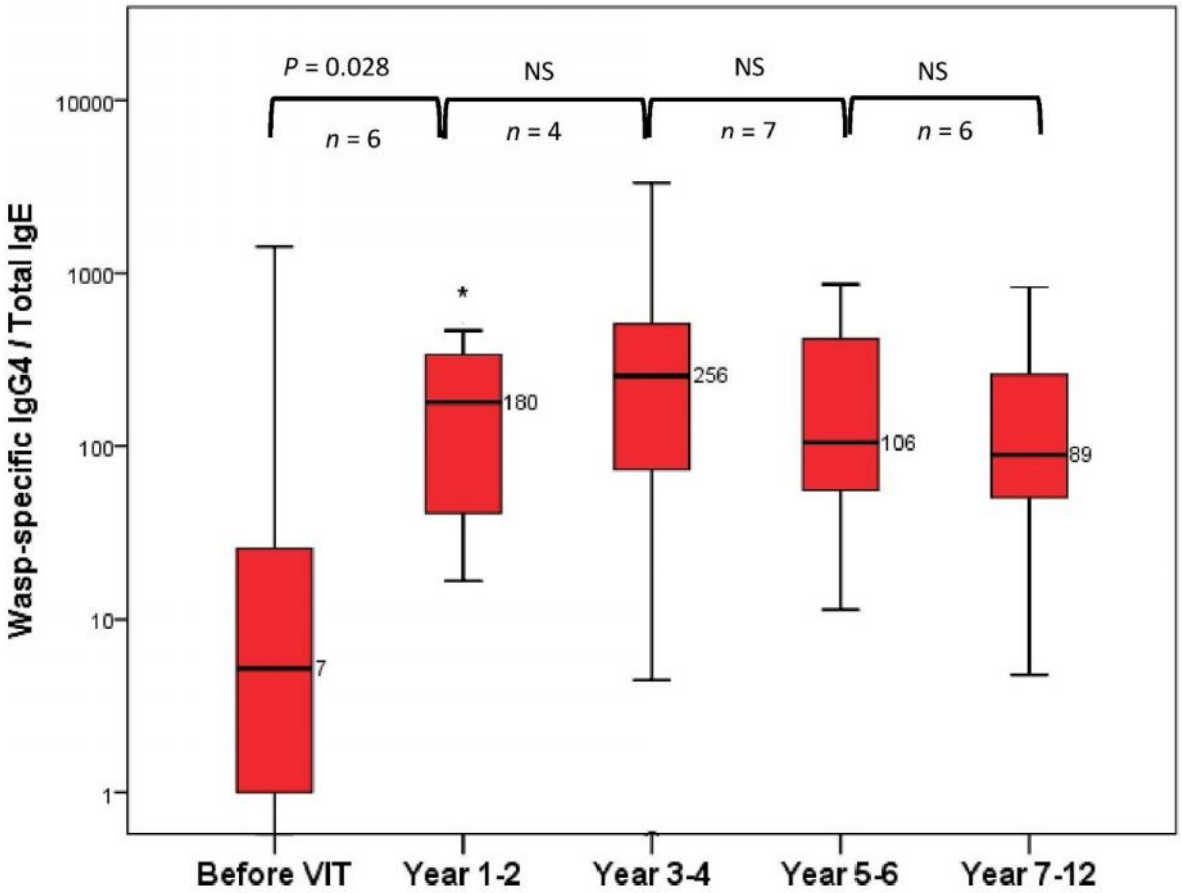
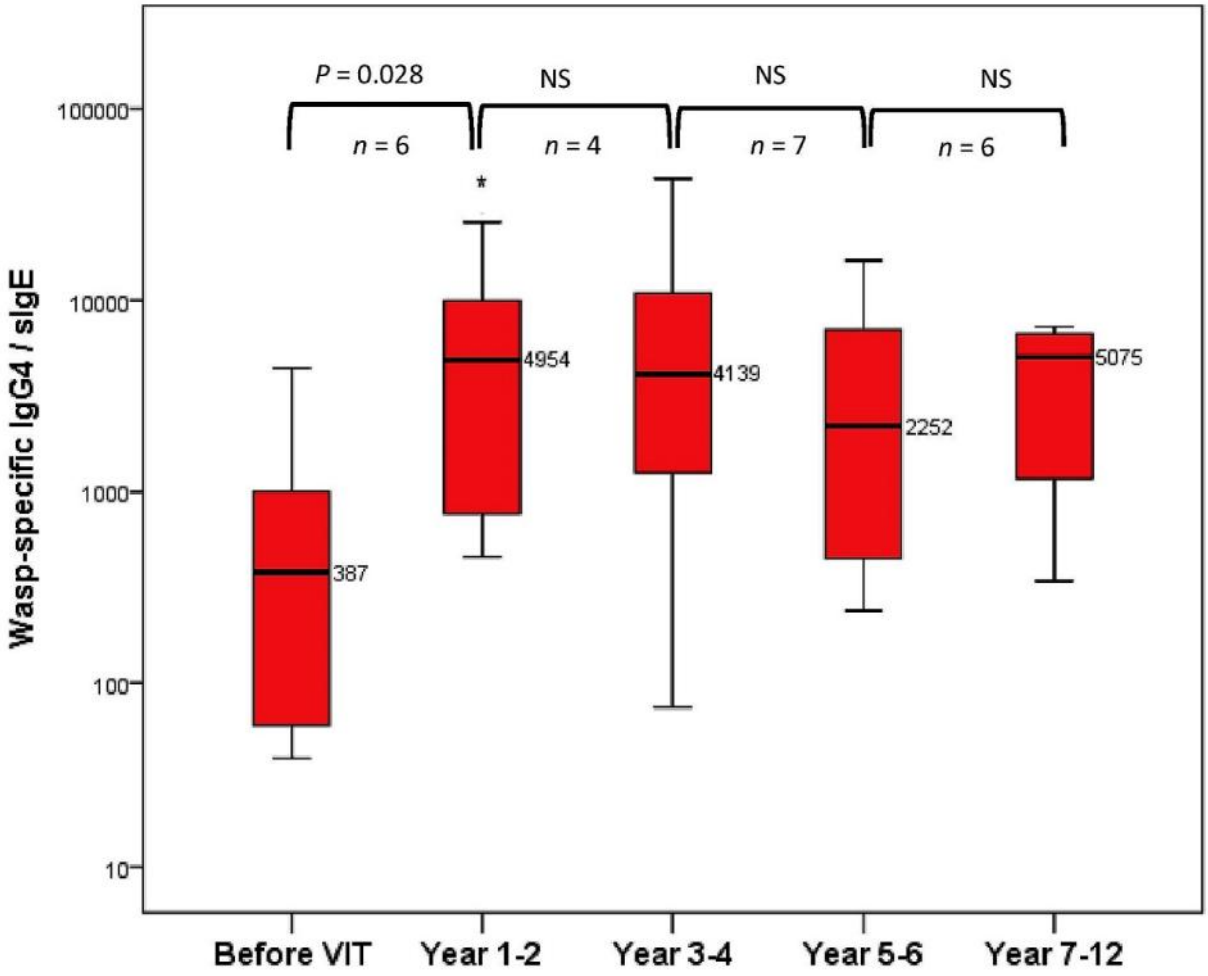

P = 0.028
NS
NS
NS
n = 6
n = 4
n = 7
n = 6
10000
1000
100
10
1
Wasp-specific IgG4 / Total IgE
*
180
256
106
89
7
Before VIT
Year 1-2
Year 3-4
Year 5-6
Year 7-12

Fig. S1C


P = 0.028
NS
NS
NS
n = 6
n = 4
n = 7
n = 6
*
100000
10000
1000
100
10
Wasp-specific IgG4 / sIgE
4954
4139
2252
5075
387
Before VIT
Year 1-2
Year 3-4
Year 5-6
Year 7-12

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112 Fig. S1D

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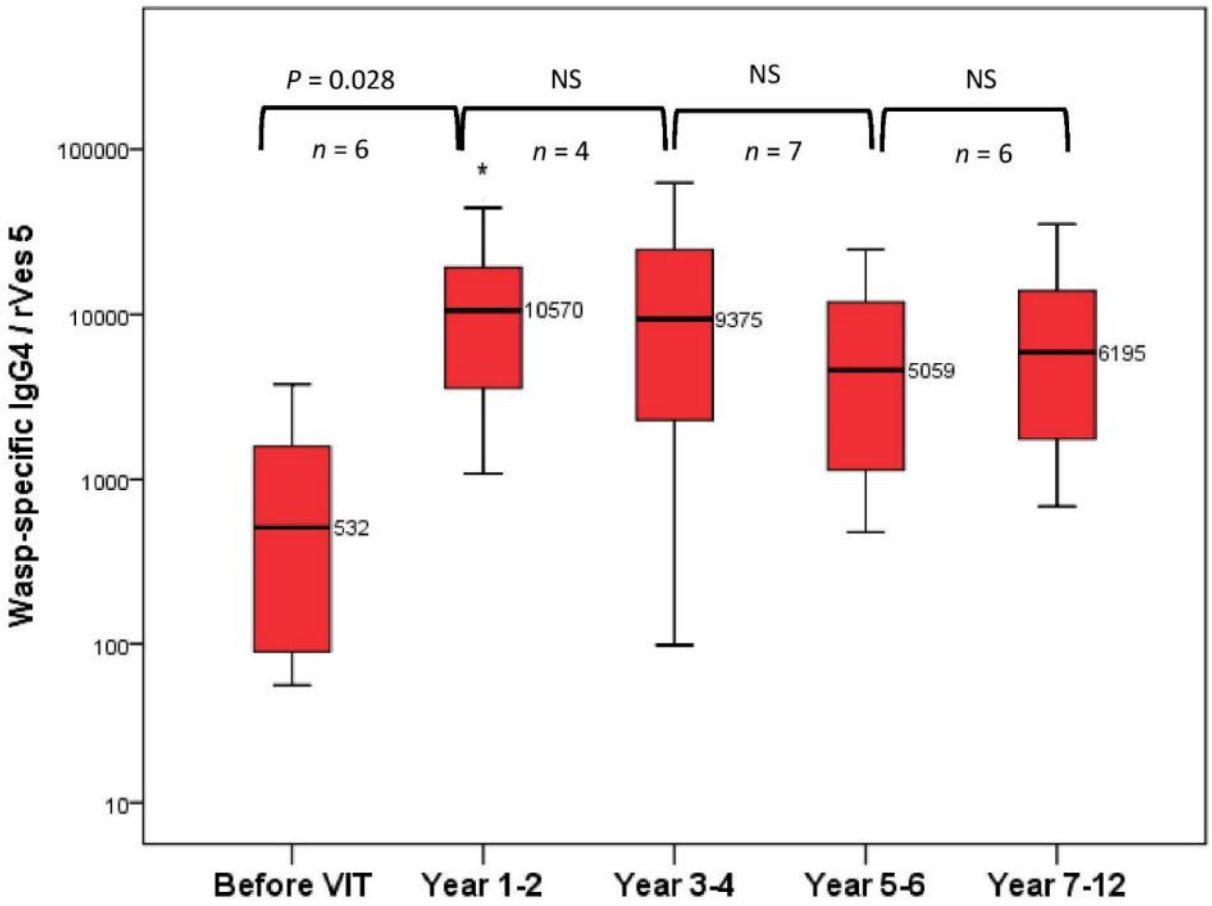

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