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Evolution of Api m10 specific IgE and IgG4 after one year of bee venom immunotherapy

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Key words

Anaphylaxis; api m1; api m10; bee venom; venom immunotherapy.

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Summary

Background. *Bee-venom (BV) anaphylaxis can be life-threatening, requiring treatment with BV immunotherapy (bVIT). Different molecular profiles may be associated with different outcomes after bVIT. Methods. In 19 patients with BV anaphylaxis, sensitized both to Api m1 and Api m10, we evaluated sIgE and sIgG4 Api m1 and Api m10 levels before and after 1 year bVIT. Results 7 patients (37%) had higher baseline Api m10 than Api m1 sIgE levels (Api m10 predominant). bVIT reduced sIgE to both components but sIgG4 levels were increased only for Api m1. 5 patients (2 in the Api m10 predominant group) were re-stung without anaphylaxis. Conclusions. Although there was no increase in Api m10 sIgG4 levels after 1 year bVIT, we did not observe relevant differences in other outcomes between patients with predominant Api m1 or Api m10 sensitization.*

Introduction

Systemic reactions to bee stings are potentially fatal in bee venom (BV) allergic patients (1). Immunotherapy with bee venom (bVIT) is a well established therapy in patients with systemic reactions although doctors of other specialties, namely by emergency physicians are not always familiar (2,3). It has been shown to improve quality of life and to prevent life-threatening reactions following an accidental sting (4).

Accepted criteria for bVIT include systemic reactions following a bee sting together with a certain degree of probability that the patient may be stung again, along with the unequivocal demonstration of a IgE-mediated reaction to bee venom, either by skin tests or serum specific IgE to whole BV extracts.

Component resolved diagnosis allows the identification of major species-specific allergens, which may contribute to a more accurate diagnosis in some patients (5). In recent years recombinant BV allergens, such as Api m4, have been associated with a higher frequency of adverse reaction to bVIT (6) or with lesser effectiveness of bVIT, and Api m10 (7).

Api m10 is a major BV allergen, that is recognized by more than 50% of BV allergic patients of different populations (8,9) and inclusively in some patients that are negative to Api m1. In an unselected population of BV allergic patients followed in our Hospital, positivity to Api m10 was present in 70%, being second only to Api m1 (positive in 86%) (9).

Since it has been reported that several bVIT extracts lack Api m10 or that is present in only very small quantities (10), the predominance of Api m10 sensitization has been proposed as a possible predictive marker of bVIT failure (7). Significant differences in Api m10 concentrations between different manufacturers and, in one case, significant differences between batches of the same manufacturer have been reported (7,11). These reports suggest differences in the quality of therapeutic BV ex-

tracts, which could also be related to different manufacturing processes, a fact that might be of major importance at least for patients with particular sensitization profiles (11).
Therefore, the aim of our work was to evaluate if BV allergic subjects, positive to Api m1 and Api m10 showed any changes in specific IgE and IgG4 to Api m10 after one year of bVIT, with a BV extract (Roxall®). According to the manufacturer, this extract contains Api m10 in an unknown quantity. As far as we know this particular BV extract was not evaluated in any of the previously published papers regarding this subject.

Material and methods

Population

Retrospective study of patients with BV anaphylaxis, grade III/IV according to Muller classification, with sIgE positivity both to Api m1 and Api m10. Patients should have completed at least one year of immunotherapy with the same commercial BV extract sera analysis before and after one year of bVIT. A total of 19 patients were evaluated, predominantly male (89%) with a mean age of 49.5 years (14-74 years).

Diagnosis of bee venom allergy

Diagnosis was based on a clinical history of recurrent anaphylaxis after a bee sting and positive skin tests and/or positive sIgE to BV whole extract. Furthermore, all patients have IgE-positive to both to Api m1 and Api m10.

Skin tests

Skin tests with BV extracts were performed according to EAACI guidelines (1) with Stallergenes® or Bial-Aristegui / Roxall® extracts, at least three weeks after the last sting reaction. The skin prick tests were performed using a 100 µg/ mL concentration and with 0.9% NaCl as the negative control and 10 mg/ml histamine as the positive control. Intradermal tests were performed with increasing concentrations from 0.001 to 1 µg/ml as well as a negative control.

Specific IgE/ IgG4 evaluation

Specific IgE antibody (sIgE) levels and specific IgG4 (sIgG4) to BV whole extract, and recombinants to Phospholipase A2 (Api m1) and Icarapin (Api m 10) were evaluated in all patients using ImmunoCAP® system according to the manufacturer's instructions (ThermoFisher Scientific, Uppsala, Sweden). Values of ≥ 0.35 kU/L for sIgE to BV and > 0.10 for sIgE to Api m1 or Api m10 were considered positive. These measurements were undertaken before and one year after start of bVIT

Venom immunotherapy ultra-rush (UR) protocol

The induction protocol used was the 210-minute UR proposed by Birnbaum (12), used by our group in the last years with a good safety profile (13). In this protocol a cumulative dose of 101.1 µg, divided in 6 injections, is administered as follows: an initial dose of 0.1 µg, followed by 1, 10 and 20 µg at 30-minutes intervals. Then 30 and 40 µg were given every 60 minutes. The maintenance dose of 100 µg was repeated 15 days after the UR and administered at 4-6-week intervals over a period of 3 to 5 years, as established by the EAACI guidelines (1). All patients received the BV extract from Bial-Aristegui / Roxall®.
All injections were given by trained medical staff in an Immunoallergology Day Hospital, equipped for the treatment of anaphylactic reactions. All patients had a venous access with saline during the procedure. Heart rate, blood pressure and peripheral oxygen saturation were continuously monitored. Patients received pretreatment with oral H1 antihistamine (cetirizine 10 mg, ebastine 10 mg or other equivalent 2nd generation non-sedating H1 antihistamine) in the 2 days prior to UR and in the morning of the UR.
Therapy with ACE inhibitors or with cardio-selective beta blockers in patients with stable cardiovascular disease was continued during UR and bVIT.
This study was approved by the Ethics Committee of the Hospital Santa Maria and was conducted according to ethical standards established in the Declaration of Helsinki. Informed consent was obtained from all participants before enrolment in the study.

Results

All individual measurements of sIgE and sIgG4 to BV, Api m1 and Api m10 are shown in **figure 1** and **table I** (before bVIT - T0) and in **table II** (after one year bVIT - T1). **Table III** shows mean, median and interquartile ranges of sIgE and sIgG4 values at T0 and T1.
In T0, the mean and the median Api m1 sIgE levels were higher than the Api m10 sIgE levels but the analysis of individual values shows that only 12 patients (63%) had higher Api m1 sIgE levels than Api m10 sIgE levels while in 7 patients (37%) the baseline Api m10 sIgE values were in fact higher. We found no differences in the age or in other characteristics between these two groups of patients. In T0 the sIgG4 values were low for both recombinants, and they were zero in the majority of patients and they did not have any correlation with sIgE values.
Figure 2 depicts individual variations of sIgE and sIgG4 values to Api m1 and Api m10, before and after one year of bVIT.
We observed reductions in Api m1 and Api m10 sIgE values, but these reductions were significant ($p<0.05$) only in the case of Api m10.

Table I *- Individual patients' values before bVIT.*

Patient no	Gender	Age	T0 (sIgE)			T0 (sIgG4)		
			BV	r Api m1	r Api m10	BV	r Api m1	r Api m10
1	M	30	92.90	61.10	8.84	0.00	0.00	0.77
2	M	60	25.60	0.94	4.71	1.66	0.10	0.00
3	F	43	6.05	5.78	0.59	0.01	0.00	0.00
4	M	55	30.00	7.34	2.75	1.37	0.53	0.00
5	M	72	24.70	0.87	0.49	0.32	0.01	0.00
6	M	56	0.39	0.39	0.12	3.08	0.00	0.00
7	M	31	4.12	1.46	1.50	0.00	0.00	0.00
8	M	64	11.20	0.16	0.73	0.00	0.00	0.00
9	M	74	3.55	0.28	2.75	3.27	3.57	0.00
10	M	66	8.99	7.17	0.14	0.00	0.00	0.00
11	M	14	100.00	90.70	11.10	6.00	0.09	0.00
12	M	43	2.71	0.60	2.64	1.03	0.74	0.00
13	M	66	11.40	7.85	0.19	0.00	0.00	0.00
14	M	39	11.10	0.65	2.82	11.20	5.18	0.00
15	M	43	16.70	18.80	2.45	2.88	2.58	0.00
16	F	48	33.00	20.90	0.34	0.00	0.00	0.00
17	M	35	23.30	31.60	6.99	0.00	0.00	0.00
18	M	60	8.99	5.42	12.60	0.00	0.00	0.00
19	M	43	1.39	0.47	0.36	0.00	0.00	0.00

BV- whole bee venom extract.

Figure 1*. – Individual sIgE and sIgG4 values to BV and recombinants before bVIT BV- whole bee venom extract.*

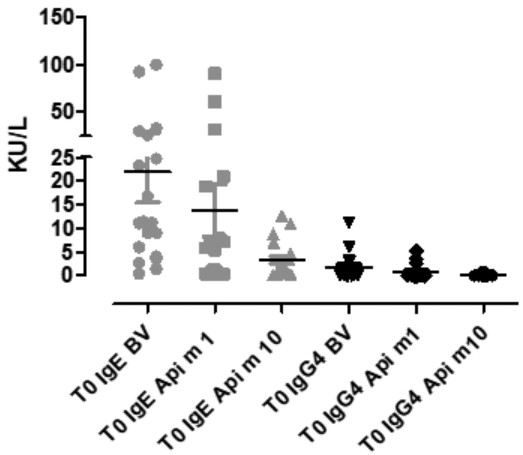


Table II *- Individual patients' values after one year of bVIT.*

Patient no	Field sting during bVIT (Yes/No)	T1 (sIgE)			T1 (sIgG4)		
		BV	r Api m1	r Api m10	BV	r Api m1	r Api m10
1	Yes	98.10	86.20	2.55	26.80	14.60	0.00
2	No	2.45	0.05	0.52	1.35	0.29	0.00
3	No	0.11	3.47	0.59	0.88	1.89	0.00
4	No	100.00	36.8	10.00	11.90	6.65	0.00
5	Yes	22.20	5.55	2.10	22.20	21.30	0.18
6	No	0.60	0.23	0.13	1.30	0.46	0.00
7	No	15.40	5.24	0.82	8.68	6.32	0.00
8	Yes	2.88	0.15	0.26	0.00	4.47	0.00
9	No	2.75	0.43	1.88	5.71	4.48	0.00
10	No	4.99	3.73	0.07	0.00	0.00	0.00
11	No	82.70	50.70	6.16	0.00	27.70	0.00
12	No	1.61	0.37	1.94	0.00	27.90	0.00
13	Yes	5.86	2.35	0.17	0.00	0.00	0.00
14	Yes	8.53	1.23	2.70	0.00	8.75	0.00
15	No	6.20	1.62	1.50	0.00	11.10	0.00
16	No	36.40	21.00	0.52	0.00	0.00	0.00
17	No	5.29	3.92	1.80	0.00	0.00	0.00
18	No	2.00	0.97	2.99	0.00	0.00	0.00
19	No	1.70	0.45	0.37	0.00	0.00	0.00

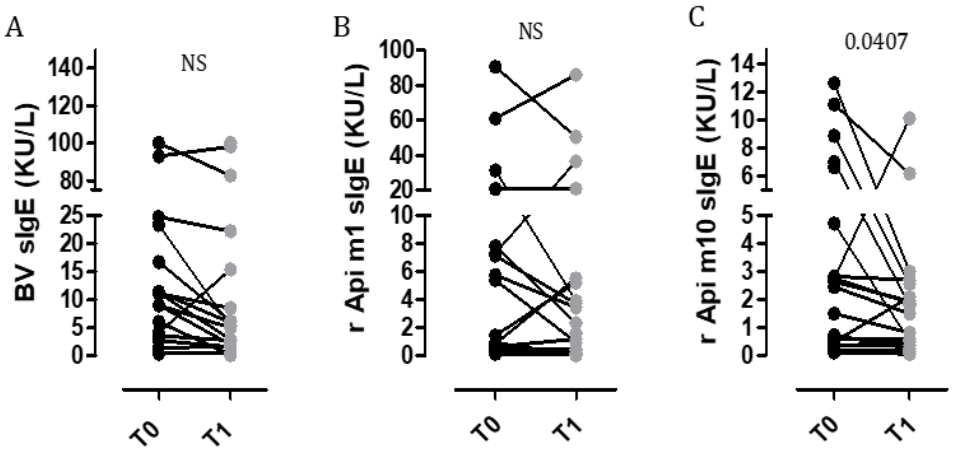
BV- whole bee venom extract.

Table III *- Mean, median and interquartile (IQR) specific immunoglobulin values before (T0) and after one year (T1) of bVIT.*

	sIgE T0			S IgE T1		
	Mean	Median	IQR25/75	Mean	Median	IQR25/75
BV	21.90	11.20	4.12/25.60	21.04	5.29	2.00/22.20
r Api m1	13.81	5.42	0.60/18.80	11.81	2.35	0.43/5.55
r Api m10	3.27	2.45	0.36/4.71	1.96	1.50	0.37/2.55
	sIgG4 T0			**S IgG4 T1**		
	Mean	Median	IQR25/75	Mean	Median	IQR25/75
Bv	2.20	1.20	0.00/3.13	7.57	1.62	0.00/14.48
r Api m1	0.91	0.05	0.00/1.20	9.72	6.49	1.53/16.28
r Api m10	0.06	0.00	0.00/0.00	0.01	0.00	0.00/0.00

BV- whole bee venom extract.

Figure 2 *– Whole bee-venom extract (A), Api m1 (B) and Api m10 (C) sIgE values of individual patients before (T0) and after one year bVIT (T1) NS – not significant BV- whole bee venom extract.*



Additionally we have also documented significant increases in Api m1 sIgG4 values but not in Api m10 sIgG4.
During this first year of bVIT, only 5 of our 19 patients were accidentally re-stung. We did not record any systemic reactions or use of adrenaline. Two of these 5 patients belonged to the group with baseline higher Api m10 sIgE than Api m1 sIgE values.

Discussion

Our results show that in these BV allergic patients with sensitization to both to Pai m1 and Api m10, one year of the bit, can induce immunologic responses to whole BV and to Apia m1 with mean singe reductions of more than 50% and significant increases (>300%) in sIgG4 to Pai m1. Besides these expected changes we have additionally shown that one year of bVIT that, according to the manufacturer, contains Api m10 in an unknown quantity, could also induce significant reductions in Api m10 specific IgE, but without any increases in Api m10 sIgG4. These results are in line with the reports by Kohler et al (14) that showed no increase in Api m10 sIgG4 levels in 20 BV-allergic patients receiving b-VIT for 12-48 months and by Frick et al (7) that showed that b-VIT with one of the three commercial extracts in which they did not detect significant amounts of Api m10 induced some significant reductions of sIgE to Api m10 but without any significant increase of sIgG4 to Api m10. On the other hand, patients treated with one of the two commercial extracts where they did detect amounts of Api m10 similar to crude venom preparations showed higher and very significant reductions in Api m10 sIgE levels as well as significant increases in sIgG4 to Api m10 (7).

Api m1 (phospholipase A2) comprises 12-15% of the dry weight of bee venom but it is the most relevant allergen present in crude venom and in venom extracts and it represents the sensitization most frequently found in bee-allergic patients (15). However, Api m1 sIgE is not always present in bee-venom allergic patients, ranging from 57 to 97% in previously published papers (14) with 86% positivity reported by our group in a prospective study of 30 portuguese bee-venom allergic patients (9). Api m1 sIgE negative patients with a clear history of bee-venom induced anaphylaxis can be a diagnostic challenge and it has been proposed that the inclusion of other bee-venom specific recombinant allergens, such as Api m3 or Api m10, in diagnostic panels could increase diagnostic sensitivity (16).
Api m10 (icarapin) comprises less than 1% of the dry weight of BV (15) but it is a major allergen. Api m10 positivity in populations of BV allergic patients has been reported to range between 49 and 62% in older studies (15), with more recent studies reporting frequencies around 70%, meaning that Api m10 is second only to Api m1 sensitisation (7,9). Furthermore, some of the Api m10 sIgE positive patients are negative to Api m1 sIgE, which raises not only diagnostic problems but also therapeutic concerns, since Api m1 is present in adequate quantities in all bVIT extracts but Api m10 is apparently underrepresented in many bVIT extracts (7,11,14), a fact that was proposed to explain treatment failures of bVIT in patients with a predominant Api m10 sensitisation (defined as a percentage of Api m10 sIgE in relation to honey bee venom sIgE > 50%) (7).
In our study we included only patients with double positivity to Api m1 and Api m10 and we observed that, in this group of patients, more than 1/3 had higher baseline sIgE values to Api

m10 than to Api m1. If we applied to our patients the definition of predominant sensitisation (sIgE to recombinant allergen>50% of sIgE to whole BV) used by Frick et al (7), we would have 9 patients with predominant Api m1 sensitisation and 3 patients with predominant Api m10 sensitisation, with the 7 remaining patients not showing any predominance with respect to Api m1 or Api m10. Independently of the way we look at it, it is a fact that patients in whom Api m10 constitutes the dominant sensitisation represent a non-negligible percentage of BV allergic patients.

All these data on the relevance of Api m10 sensitized patients have generated some debate whether particular BV sensitization profiles are related to better or worse outcomes of bVIT (7). It has also been suggested that, in a personalised medical approach, patients with a predominant Api m10 sensitisation should receive a bee-venom extract containing adequate amounts of Api m10 and that patients without Api m10 sensitisation should receive a bee-venom extract with low or absent Api m10 (11).

In Portugal we do not usually perform controlled sting challenges and the evaluation of the effectiveness relies mainly on patients reporting what happened when they were re-stung. In this study more than 25% of the patients were re-stung during bVIT and no one reported any systemic reaction or use of adrenaline following accidental stings, independently of the predominant sensitisation they had. This finding, that does not agree with the report by Frick et al (7), should be interpreted with caution because of the very small number of patients involved and the non-controlled nature of the observation.

The present study has clear limitations in that it used a retrospective study design with a limited number of patients. Also, the quantity of Api m10 in the BV extract used is unknown. But this study has the added interest of reporting individual immunological data obtained by the same BV extract, one that has not been addressed in previous studies focusing on the importance of Api m10 sensitisation profiles and Api m10 content in commercial BV extracts. Additionally, our data were obtained from a well-characterized population of BV allergic patients with anaphylaxis, showing double positivity to the two more prevalent recombinant allergens in our Portuguese BV allergic patients: Api m1 and Api m10.

We hope that our paper as well as other studies could stimulate a more in-depth and widespread knowledge of the full spectrum or recombinant allergens present in each of the different BV commercial extracts, since this knowledge could have potentially vital implications in therapeutic options for severe honey-bee venom allergic patients.

Conclusions

In our group of 19 BV-allergic patients with anaphylaxis to BV and with double positivity to Api m1 and Api m10, one year of bVIT induced reductions of Api m1 and Api m10 sIgE levels but only significant increases of Api m1 sIgG4 levels and not of Api m10. According to the manufacturer, this BV extract contains an unknown quantity of Api m10 allergen and it is possible that the Api m10 concentration present in the extract is not sufficient to induce sIgG4 responses. However, from a clinical point of view we did not observe any systemic reactions in re-stung patients, therefore suggesting clinical efficacy of this BV extract, even in Api m10 sensitised patients.

Further studies are needed to compare the relative Api m10 concentrations in all the different commercial BV extracts and to compare immunologic and clinical efficacy of bVIT with different extracts in patients with different sensitization profiles.

Conflict of interests

The authors declare that they have no conflict of interests.

Author contributions

MCPS, EP, MBF, MPB designed research; MCPS, TL, MBF, performed research and analyzed data; EP and MPB were involved in clinical investigation of patients; MCPS and MBF wrote the paper.

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