Molecular Profiling in Bee Venom Allergy – clinical and therapeutic characterization in a Portuguese cohort

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Abstract

Introduction: Bee venom allergy (BVA) can trigger local and systemic allergic reactions, including anaphylaxis. Recently, the molecular sensitization profile has gained importance in the reaction's stratification and venom immunotherapy (VIT).

Methods: Retrospective analysis of patients with hypersensitivity to BVA, confirmed by sIgE to *Apis mellifera* ≥0.35 kU/L and/or positive skin tests to bee venom commercial extract, evaluated in specialized consultation. Demographic, clinical, and laboratory data (including molecular *Api m 1, 4, and 10*) were

analyzed, looking for risk factors associated with the severity of the index reaction and reactions during VIT.

Results: 93 patients were included (55.9% male; median age of 46 years), 57.3% with atopic comorbidities, and 23.4% with cardiovascular comorbidities. The median specific IgE to *Apis mellifera* was 6.7 (IQR 1.0-20.3) kU/L. Regarding the molecular profile, the median IgE to *Api m 1* was 0.5 kU/L (57.5% positive out of all measurements); *Api m 4* - 0.01 kU/L (11.9% positive), and *Api m 10* - 0.3 kU/L (50.0% positive). No patient was monosensitized to *Api m 4*. The median age of the most severe sting reaction was 36 (IQR 26-48) years, with a median severity (Müeller scale) of 3 (IQR 2-3). Forty-seven patients (50.5%) underwent VIT, with 35.6% of reactions recorded. The severity of the index reaction correlated positively with older ages (p=0.040; r=0.249), in contrast to monosensitization to *Api m 1*, which was an independent predictor of milder reactions (p=0.015). Sensitization to *Api m 10* was associated with a higher likelihood of reactions during VIT (p=0.038) but potentially less systemic reactions at re-stings (p=0.097).

Conclusions: Molecular sensitization profile appears to be relevant not only to the severity of index reactions but also during VIT. Studies of a large cohort of patients with molecular profiles are essential to validate these results and improve the clinical and therapeutic approach to BVA.

Key words: bee venom allergy; venom immunotherapy; component-resolved diagnostics;

Impact statement: Molecular allergens to *Apis mellifera* appear to be useful in stratifying severity of bee venom allergy index reactions but also in predicting the efficacy and safety of venom immunotherapy in a Portuguese population.

LIST OF ABBREVIATIONS

BVA: bee venom allergy

IQR: interquartile range

LLR: large local reactions

SSR: systemic sting reactions

VIT: venom immunotherapy

WHO/IUIS: World Health Organization and International Union of Immunological Societies

INTRODUCTION

Insect stings by hymenoptera species such as honeybees are very common, with data indicating that 56.6%-94.5% of the general population has been stung at least once in their lifetime (1).

The most frequent clinical presentations of bee venom allergy (BVA) are large local reactions (LLR) at the sting site and systemic sting reactions (SSR). A LLR has been defined as a swelling exceeding a diameter of 10 cm that lasts for longer than 24 h (2). In SSR, mild symptoms usually manifest as generalized skin conditions including flushing, urticaria, and angioedema. Typically, dizziness,

dyspnea, and nausea are examples of moderate reactions, while shock and loss of consciousness, or even cardiac or respiratory arrest, define a severe SSR. Severe reactions are life threatening and have been attributed to fatalities. The rate of self-reported SSR in European epidemiological studies ranges from 0.3 to 7.5% in adults (3), while LLRs occur in 2.4% to 26.4% of the general population (4).

The only treatment that can potentially prevent further SSR is venom immunotherapy (VIT), which is reported to be effective in 77 to 84% of patients treated with honeybee venom (5,6). It is known that specific immunotherapy with bee venom versus wasp venom is usually associated with lower therapeutic efficacy and a higher risk of systemic reactions during treatment (7). It is therefore extremely important to identify potential biomarkers for assessing therapeutic efficacy and severity.

A total of 12 allergenic fractions from the honeybee (*Apis mellifera*) are known and registered, and they can be found in the official database of allergens of the WHO/IUIS Allergen Nomenclature Sub-Committee (8). As many as 11 of these allergens come from bee venom (*Api m 1-10*, *Api m 12*), while two allergenic isoforms are derived from bee secretions from the royal jelly-producing glands (*Api m 11a* [0101] and *Api m 11b* [0201]).

Currently, commercially available hymenoptera allergens for component resolved allergy testing include *rApi m 1* (phospholipase A2), *rApi m 2* (hyaluronidase), *rApi m 3* (a venom acid phosphatase Acph-1), *rApi m 4* (a melittin), *rApi m 5* (a Dipeptidylpeptidase IV) and *rApi m 10* (an icarapine) for honeybee venom.

In addition to their already recognized role in the proper diagnosis of BVA patients, the molecular components of bee venom can play an important role in

identifying potential cross-reactivity, as well as an important role as markers in assessing efficacy and severity (9).

Some components are better characterized in the literature than others, such as *Api m 1* (a major bee venom allergen) and *Api m 2* (considered a marker of cross-reactivity), while others have yet to be fully studied. Some studies associate sensitization to *Api m 10* with less effective immunotherapy (10,11) and lower tolerance to immunotherapy in patients sensitized to *Api m 4* – a minor allergen in the venom but with a high percentage of dry weight (12,13).

Thus, characterizing the molecular sensitization profile has become increasingly important in stratifying the severity of reactions to stings, as well as in the efficacy of VIT and in predicting adverse reactions throughout treatment. We decided to characterize the clinical and laboratory profiles of BVA patients in a Mediterranean cohort.

MATERIAL AND METHODS

Study design

This cross-sectional study was conducted in the Allergy and Clinical Immunology Unit of a tertiary hospital in Portugal. We included patients that were followed-up at our outpatient clinic between January/2012 and July/2023 and were sensitized to bee venom. Sensitization was defined as having serum specific IgE (sIgE) to *Apis mellifera* venom ≥0.35 kU/L and/or positive skin prick/intradermal tests to *Apis mellifera* venom (Roxall Medicina, Spain). Subsequently, we collected demographic, clinical and additional laboratory data using electronic hospital records (*SClínico)* and national health registry (*Registo de Saúde Eletrónico – RSE)*.

Participants

A total number of 93 allergic patients were enrolled in this study. Patients had to meet two criteria to be classified as being allergic to bee venom – bee venom sensitization and reported systemic symptoms after bee sting.

Data collection

The collected data included demographic, clinical and laboratory variables.

Patient's gender, age at index-reaction, age at time of data collection and beekeeping-related occupations were selected as our demographic variables.

Index-reaction was characterized as the most severe among the earliest sting reactions in BVA patients. Farmers and hobbyists associated with beekeeping were also defined.

Clinical data comprised of characterization of atopic and cardiovascular comorbidities, severity and number of stings during index-reaction and bee VIT. Data was registered in a case report form, based on electronic registries. The clinical background of atopic comorbidities was assessed individually for asthma, allergic rhinitis and food allergy. Arterial hypertension, diabetes, ischemic cardiopathy, dyslipidemia and obesity were considered cardiovascular comorbidities. Information about the severity of the index allergic reaction was collected and stratified according to the Mueller classification of systemic reactions to insect stings (2,14). Severity ranged from grade 1 (urticaria, itching, malaise, and anxiety) to the more severe grade 4 (mucocutaneous, respiratory and/or gastrointestinal symptoms, plus two or more of the following: fall in blood pressure, collapse, loss of consciousness, incontinence, cyanosis). A cluster protocol for VIT patients was performed in our allergy center. Patients that

concluded or were still undergoing bee VIT were also characterized according to the occurrence of systemic reactions during immunotherapy and/or with re-stings.

The following laboratory data was collected: basal tryptase (Thermo Fisher Scientific, United States), *Apis mellifera* venom sIgE and venom component sIgE (ImmunoCAP, Thermo Fisher Scientific, United States), at the time of the first observation at our clinic. For VIT patients, sIgE to *Apis Mellifera* is expected to vary over time. As such, it was collected in two time periods – before and more than 12 months after VIT. *Apis mellifera* and venom component sIgE were considered positive when levels were ≥0.35 kU/L. Component-specific sIgE included the allergens *Api m 1, Api m 4* and *Api m 10*.

Statistical analysis

All statistical analyses were done using STATA software (version 16.1, StataCorp LLC, Texas, USA) in order to assess for correlations between the severity of systemic index reactions and demographic, clinical and laboratory data. Patient characteristics were described as a percentage for categorical data and either as mean (standard deviation) or median (interquartile range) for continuous data, depending on observation of normality. Normality was assessed through histogram interpretation. P-values of <0.05 were considered statistically significant. Chi-squared or Exact Fisher tests were used for correlation between categorical variables. Correlation between continuous and categorical data was assessed using t-test or Mann-Whitney U, depending on observation of normality. Multivariate linear regression was used to control for confounding.

RESULTS

Patient characteristics at allergy onset

Our study included 93 BVA patients. The median age at index-reactions was 36 (IQR 26-48) years, and 52 (55.9%) were males. Forty (43.0%) patients were associated with beekeeping activities. Atopic comorbidities were highly prevalent (57.3% of 75 patients), whereas roughly a quarter (23.4% of 77 patients) presented with cardiovascular comorbidities. No patients were diagnosed with mast-cell diseases or hereditary alpha tryptasemia.

Eighty-five patients with BVA reported index-reactions with a median severity of 3 (*IQR 2-3*), with most (n=53, 62.4%) presenting with highly severe reactions (grades 3 and 4). Forty patients were able to discriminate the number of bee stings, with 30 (75.0%) reporting a single one. The clinical characteristics and demographics of the study participants are depicted in **Table I**.

Bee venom immunotherapy

In our cohort, 47 (50.5%) patients underwent VIT. Patients who did not have VIT mainly refused treatment, and a few were contraindicated for it (due to pregnancy or active malignancy). Treatment was completed (median of 5 [IQR 5-6] years) in 19 (40.4%), 2 patients (4.2%) abandoned treatment (one due to loss to follow-up and the other discontinued after several systemic reactions to VIT) and 26 (55.4%) are still under treatment. Sixteen patients (35.6%) reported systemic reactions during immunotherapy (mostly grades 1 and 2). Additionally, 25 (55.4%) patients were re-stung, of which only four reported SSR. Most of these re-stung patients were related to beekeeping activities (n=18, 72.0%).

Laboratory data

The median (IQR) *Apis mellifera* sIgE levels of our cohort were 6.7 (1.0-20.3) kU/L. In our sub-group of VIT patients, pre-treatment levels were higher (13.7; 5.3-43.5), dropping significantly during follow-up to 3.3 (1.1-7.9) kU/L.

Regarding molecular sIgE sensitization, patients were tested for *Api m 1* (n=73)*, Api m 4* (n=42) and *Api m 10* (n=64). Overall, *Api m 1* was positive in 42 patients (57.5%) and *Api m 10* in 32 (50.0%), whereas only 5 patients (11.9%) had positive *Api m 4* levels. Three (5.3%) patients of all cohort were sensitized to all three allergens. Median (IQR) sIgE levels for *Api m 1, 4* and *10* were 0.47 *(*0.10-1.57*),* 0.01 *(*0.00-0.08*)* and 0.32 (0.08-1.62), respectively.

In the VIT subgroup, among patients whose molecular components were available, 18 (72.0% of 25 measurements) were sensitized to *Api m 1*, and 15 (65.2% of 23) to *Api m 10*, in contrast with only 2 (10.5% of 19) patients with positive sIgE to *Api m 4.* These same two patients were also polysensitized to the other molecular allergens. Detailed sensitizations to molecular allergens are depicted in **Tables I.**

Associations between systemic reactions and demographic, clinical or laboratory variables

1. *Severity of index-reaction to bee sting was associated with age and molecular sensitization profiles to Apis mellifera*

Severity of the reaction at allergy onset was classified in 85 patients. There was a weak but significant positive correlation with age at onset, with older patients presenting more severe reactions (Spearman's coefficient [*rho*]=0.249*, p*=0.040). Inversely, patients that were only sensitized to *Api m 1* (among the three

measured proteins) had milder reactions in comparison to non-monosensitized patients – 2 (*IQR* 2-2*)* vs 3 (*IQR* 3-4), p=0.015. When fitted in a multivariate linear regression model that included variables with *p*<0.100 (age, atopic comorbidities and sensitization solely to *Api m 1*) and used a backward stepwise approach, the *Api m 1* mono-sensitization profile retained its significance (*p*=0.031) and was considered an independent predictor for milder systemic index-reactions to bee sting. Statistical analysis for all demographic, clinical and laboratory variables is described in **Table II.**

2. *Occurrence of systemic reactions during VIT was associated with sensitization to Api m 10*

Patients that concluded or were still undergoing VIT were analyzed. No significant associations were found between demography, clinical and laboratory variables and the proportion of VIT patients with systemic reactions, with one notable exception – patients sensitized to *Api m 10*, regardless of potential co-sensitizations, were significantly more associated with systemic adverse reactions during VIT when compared to non-systemic reactions (90.0 *vs* 46.2%, *p=0.038*). Statistical analysis for potential associations is depicted in **Table III.**

3. *Absence of systemic reactions with bee re-stings was potentially associated with sensitization to Api m 10 in VIT patients*

In the VIT subgroup, no statistically significant associations were found between systemic reactions to re-stings and molecular sensitization profiles. However, re-stung patients non-sensitized to *Api m 10*, regardless of potential co-sensitizations, had a tendency for association with systemic adverse reactions

(25.0 vs 85.7%, *p*=0.093). Additionally, lower levels of *Api m 10* also appeared to be marginally associated with systemic reactions in re-stings (0.15 vs 1.08 kU/l, *p*=0.059). Inversely, *Api m 4* sensitization (in addition to the other two molecular allergens) was marginally associated with systemic reactions (66.7 vs 0.00%, *p*=0.087), but there was no association with sIgE levels. Statistical analysis for potential associations is summarized in **Table IV.**

DISCUSSION

Our study aimed to characterize the clinical and laboratory profiles of a Portuguese cohort of BVA patients. We also sought to establish a relationship between molecular allergic profiles with index reactions' severity, VIT efficacy, and adverse events such as reactions during VIT and re-stings.

Several similarities were shared between our cohort and previously published studies, but there are also notable differences.

Regarding demographic and clinical background, most of our patients were young male beekeepers, which is known to fit with the national profile and is also the occupational activity most commonly associated with BVA (15). Atopic comorbidities were highly prevalent, which has also been observed in other cohorts (16). No patients were diagnosed with mast-cell diseases nor had elevated basal tryptase levels, which are known predisposing factors for anaphylaxis to hymenoptera, but were absent in our cohort (17).

As for molecular allergen profiling, it should be noted that at least half our patients were sensitized to *Api m 1* and/or *Api m 10*, highlighting their importance as major honeybee venom allergens (18–20). Sensitization to *Api m 4*, on the other hand,

was far less common in our cohort (11.9% of measurements). Despite *Api m 4* being mainly defined as a minor allergen, recent studies have reported a higher prevalence of this allergen in comparison with our results (12,13,21). Our acquisition of *Api m 4* for ImmunoCAP measurement has been very recent and, therefore, much of this data was obtained significantly after index-reaction, which may have influenced results. Additionally, some studies reporting higher prevalences have used alternative detection methods, such as Western Blot or ADVIA-Centaur sIgE measurement (13,21).

Index-reactions to bee stings, in most cases, were highly severe (Mueller grades 3 and 4). Severity of index-reaction appeared to be associated with older age, which has already been supported by previous studies (22) and could be explained by a larger proportion of comorbidities in these patients and its co-factorial influence on reaction severity. However, findings supporting this explanation have been contradicting, and less than a quarter of our patients reported cardiovascular comorbidities, with this variable being non-significant (22,23).

Conversely, another interesting finding is that patients monosensitized to *Api m 1* presented with milder reactions in our multivariate model. Studies assessing sting reactions' severity and *Api m 1* sensitizations have conflicting results.

Api m 1 sIgE levels did not correlate with the severity of index-reactions in previous reports (24,25). However, co-sensitization with *Api m 10* has been linked with severe reactions (21,25), which could help explain why our patients that were not sensitized to both allergens presented with milder systemic reactions.

Only half of our BVA patients underwent VIT. Even though a few patients were contraindicated for it (e.g. pregnancy, active autoimmune diseases), most declined treatment due to not being able to support costs. This has been explained in recent national studies that report the high economic burden that non-reimbursement of immunotherapy has for patients (26).

Sixteen patients (35.6%) reported systemic reactions during immunotherapy, which were globally milder than index-reactions. Although this proportion appears to be higher than in some studies (27,28), it is not largely different than national studies that used similar vaccine manufacturers (29). Treatment protocols and allergen composition could influence the occurrence of reactions during VIT (30). Our patients were treated with a cluster protocol and with aqueous extracts purified from Hymenoptera venom (Roxall Medicina, Spain). Even though sensitization to *Api m 10* appeared to be the single factor in our cohort associated with systemic adverse reactions during VIT, it could subsequently have a protective role in preventing SSR, according to our analysis of re-stung patients. Out of 47 patients undergoing VIT, twenty-five (53.2%) had re-stings. Only four re-stung patients reported SSR – an 84.0% honeybee VIT efficacy, which is in line with the literature (31). It should be noted that only half of our patients were re-stung, stressing the importance of active preventive measures during contact with hymenoptera, particularly in beekeeping activities (e.g. strengthening of body suit protection).

Despite the low number of analyzed patients, some factors related to molecular sensitization were marginally associated with the VIT efficacy. Particularly, *Api m 10* sensitization and higher *Api m 10* sIgE levels could be associated with local

re-sting reactions. This appears to contradict the results of a 2016 study in a Northern European cohort, which hypothesized that their VIT was not enriched with *Api m 10* (32). However, recently published studies in Mediterranean cohort and using similar vaccine manufacturers have hinted at the efficacy of VIT in reducing *Api m 10* levels and the severity of re-stings (20). Additionally, the composition of the Roxall vaccine is known to contain *Api m 10*. This could help explain our results, with patients sensitized to *Api m 10* having systemic reactions during the early stages of VIT but subsequently attaining tolerance to re-stings. Inversely, sensitization to *Api m 4*, despite being observed in very few patients that were also polysensitized, was marginally associated with SSR. *Api m 4* was not detected in the Roxall vaccine composition, which could explain the inefficacy of VIT observed in these patients. Recent studies also appear to corroborate our findings, reporting systemic reactions during VIT and lower efficacy during sting challenge in patients sensitized to *Api m 4* (13).

There are some limitations to be considered in this study. Its retrospective properties and dependency on clinical records could hinder the quality of collected data, especially regarding clinical characteristics such as cardiovascular comorbidities. Secondly, sIgE to molecular components were assessed according to the clinical history and routine diagnosis, but not in a systematic manner in every patient. In particular, *Api m 4* was only recently available, leading to a lack of measurements at baseline, which prevented a deeper multivariate sIgE analysis and a thorough analysis of molecular sIgE. Lastly, the low number of patients that were re-stung has also limited statistical power for potential associations. This should be properly addressed in

prospective studies focused on data gathering, larger cohorts and patient follow-up.

Despite these limitations, we conclude that elderly patients had more severe index reactions, monosensitization to *Api m 1* predicted milder reactions, sensitization to *Api m 10* was associated with a higher likelihood of reactions during VIT but potentially less systemic reactions at re-stings. Molecular sensitization appears to be relevant not only in stratifying the severity of index reactions but also in assessing VIT safety and efficacy. Studies with bigger BVA and VIT cohorts, as well a systematic molecular profiling of patients, are essential to validate these results and improve the clinical and therapeutic approach to BVA.

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JCL: Conceptualization, data curation, formal analysis, investigation, methodology, Writing – original draft, Writing - review & editing

PBA: Conceptualization, data curation, formal analysis, investigation, methodology, Writing – original draft, Writing - review & editing

HPP: Data curation, Writing - review & editing

FC: Data curation, Software, Writing - review & editing

ICF: Data curation**,** writing - review & editing

AM: Methodoloy, Data curation, Supervision and Validation

RC: Methodology, Data curation, Supervision and Validation

GC: Supervision and Validation

AT: Supervision and Validation

BT: Conceptualization, Project administration**,** Supervision and Validation

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References:

1. Yuan IH, Golden DBK. Wings and stings: Hymenoptera on vacation. Ann Allergy Asthma Immunol. 2023 Apr;130(4):429-437. doi: 10.1016/j.anai.2023.01.017.
2. Matysiak J, Matysiak J, Bręborowicz A, Kokot ZJ. Diagnosis of hymenoptera venom allergy--with special emphasis on honeybee (Apis mellifera) venom allergy. Ann Agric Environ Med. 2013;20(4):875-9.
3. Bilò BM, Bonifazi F. Epidemiology of insect-venom anaphylaxis. Curr Opin Allergy Clin Immunol. 2008;8:330–337. doi: 10.1097/ACI.0b013e32830638c5.
4. Bilò MB, Bonifazi F. The natural history and epidemiology of insect venom allergy: clinical implications. Clin Exp Allergy. 2009;39:1467–1476. doi: 10.1111/j.1365-2222.2009.03324.x.
5. Muller U, Helbling A, Berchtold E. Immunotherapy with honeybee venom and yellow jacket venom is different regarding efficacy and safety. J Allergy Clin Immunol. 1992;89:529–535. doi: 10.1016/0091-6749(92)90319-W.
6. Ruëff F, Vos B, Oude Elberink J, Bender A, Chatelain R, Dugas-Breit S, et al. Predictors of clinical effectiveness of Hymenoptera venom immunotherapy. Clin Exp Allergy. 2014;44:736–746. doi: 10.1111/cea.12275.
7. Kołaczek A, Skorupa D, Antczak-Marczak M, Kuna P, Kupczyk M. Safety and efficacy of venom immunotherapy: a real life study. Adv Dermatology Allergol. 2017;2:159–167. doi: 10.5114/ada.2017.67082.
8. WHO/IUIS Allergen Nomenclature Home Page. Available from: http://www.allergen.org/index.php.
9. Matysiak J, Matuszewska E, Packi K, Klupczyńska-Gabryszak A. Diagnosis of Hymenoptera Venom Allergy: State of the Art, Challenges, and Perspectives. Biomedicines. 2022;10:2170. doi: 10.3390/biomedicines10092170.
10. Jakob T, Rauber MM, Perez-Riverol A, Spillner E, Blank S. The Honeybee Venom Major Allergen Api m 10 (Icarapin) and Its Role in Diagnostics and Treatment of Hymenoptera Venom Allergy. Curr Allergy Asthma Rep. 2020;20:48. doi: 10.1007/s11882-020-00943-3.
11. Blank S, Seismann H, Michel Y, McIntyre M, Cifuentes L, Braren I, et al. Api m 10, a genuine A. mellifera venom allergen, is clinically relevant but underrepresented in therapeutic extracts. Allergy. 2011;66:1322–1329. doi: 10.1111/j.1398-9995.2011.02667.x.
12. Hirata H, Sato K, Ogasawara T, Funakoshi T, Shima D, Tatewaki M, et al. Sensitization to Api m 1, Api m 2, and Api m 4 in Japanese beekeepers who had experienced systemic reactions to honeybee stings. Allergol Int. 2019;68:261–263. doi: 10.1016/j.alit.2018.08.008.

13. Ruiz B, Serrano P, Moreno C. IgE-Api m 4 Is Useful for Identifying a Particular Phenotype of Bee Venom Allergy. J Investig Allergol Clin Immunol. 2016;26:355–361. doi: 10.18176/jiaci.0053.

14. Mueller HL. Diagnosis and Treatment of Insect Sensitivity. J Asthma Res. 1966;3:331–333. doi: 10.3109/02770906609106941.

15. Bousquet J, Ménardo JL, Velasquez G, Michel FB. Systemic reactions during maintenance immunotherapy with honey bee venom. Ann Allergy. 1988;61:63–68.

16. Ediger D, Terzioglu K, Ozturk RT. Venom allergy, risk factors for systemic reactions and the knowledge levels among Turkish beekeepers. Asia Pac Allergy. 2018;8:e15. doi: 10.5415/apallergy.2018.8.e15.

17. O'Connell MP, Lyons JJ. Hymenoptera venom-induced anaphylaxis and hereditary alpha-tryptasemia. Curr Opin Allergy Clin Immunol. 2020;20:431–437. doi: 10.1097/ACI.0000000000000678.

18. Ollert M, Blank S. Anaphylaxis to insect venom allergens: role of molecular diagnostics. Curr Allergy Asthma Rep. 2015;15:26. doi: 10.1007/s11882-015-0527-z.

19. Vega-Castro A, Rodríguez-Gil D, Martínez-Gomariz M, Gallego R, Peña MI, Palacios R. Api m 6 and Api m 10 as Major Allergens in Patients With Honeybee Venom Allergy. J Investig Allergol Clin Immunol. 2022;32:116–123. doi: 10.18176/jiaci.0639.

20. Pereira Santos MC, Lourenco T, Pereira Barbosa M, Branco Ferreira M. Evolution of Api m10 Specific IgE and IgG4 After One Year of Bee Venom Immunotherapy. Eur Ann Allergy Clin Immunol. 2020; doi: 10.23822/EurAnnACI.1764-1489.131.

21. Coutinho C, Pineda F, Castillo M, Pedro E, Pereira-Santos MC. Characterization of the sensitization profile of bee sting allergic patients - Association with severity of the reaction? Arq Asmas Alerg e Imunol. 2020;4. doi: 10.5935/2526-5393.20200069.

22. Chapsa M, Roensch H, Langner M, Beissert S, Bauer A. Predictors of severe anaphylaxis in Hymenoptera venom allergy: The importance of absence of urticaria and angioedema. Ann Allergy Asthma Immunol . 2020;125:72–77. doi: 10.1016/j.anai.2020.03.007.

23. Müller UR. [Hymenoptera venom anaphylaxis and cardiovascular disease]. Hautarzt. 2008;59:206, 208–211. doi: 10.1007/s00105-008-1469-3.

24. Rosiek-Biegus M, Pawłowicz R, Kopeć A, Kosińska M, Wrześniak M, Nittner-Marszalska M. Component-Resolved Evaluation of the Risk and Success of Immunotherapy in Bee Venom Allergic Patients. J Clin Med. 2022;11. doi: 10.3390/jcm11061677.

25. Gattinger P, Lupinek C, Kalogiros L, Silar M, Zidarn M, Korosec P, et al. The culprit insect but not severity of allergic reactions to bee and wasp venom can be determined by molecular diagnosis. PLoS One. 2018;13:e0199250. doi: 10.1371/journal.pone.0199250.

26. Carneiro-Leão L, Amaral L, Coimbra A. Reasons for Declining Venom Immunotherapy. Acta Med Port. 2018;31:618–623. doi: 10.20344/amp.9695.

27. Incorvaia C, Frati F, Dell'Albani I, Robino A, Cattaneo E, Mauro M, et al. Safety of hymenoptera venom immunotherapy: a systematic review. Expert Opin Pharmacother. 2011;12:2527–2532. doi: 10.1517/14656566.2011.616494.

28. Stock R, Fischer T, Aßmus K, Zoeller N, Ackermann H, Kaufmann R, et al. Safety and tolerability of venom immunotherapy: Evaluation of 581 rush- and ultra-rush induction protocols (safety of rush and ultra-rush venom immunotherapy). World Allergy Organ J. 2020 Dec 15;14(1):100496. doi: 10.1016/j.waojou.2020.100496.

29. Cosme J, Spínola-Santos A, Pereira-Santos MC, Pereira-Barbosa M. Allergic reactions after vaccination: translating guidelines into clinical practice. Eur Ann Allergy Clin Immunol. 2019;51:122. doi: 10.23822/EurAnnACI.1764-1489.85.

30. Gonzalez Guzman LA, García Robaina JC, Barrios Recio J, Escudero Arias E, Liñares Mata T, Cervera Aznar R, et al. Real-World Safety and Efficacy Clinical Data of an Improved Allergen-Specific Immunotherapy Product for the Treatment of Bee Venom Allergy. Vaccines. 2023;11:979. doi: 10.3390/vaccines11050979.

31. Sahiner UM, Durham SR. Hymenoptera Venom Allergy: How Does Venom Immunotherapy Prevent Anaphylaxis From Bee and Wasp Stings? Front Immunol . 2019;10. doi: 10.3389/fimmu.2019.01959.

32. Frick M, Fischer J, Helbling A, Ruëff F, Wieczorek D, Ollert M, et al. Predominant Api m 10 sensitization as risk factor for treatment failure in honey bee venom immunotherapy. J Allergy Clin Immunol. 2016;138:1663-1671.e9. doi: 10.1016/j.jaci.2016.04.024.

Table I: Characteristics of patients with bee sting reactions (n=93)

Variables	Values	Total patients
Demographic data		
Current age	46 (34-55)	93
Male gender, n (%)	52 (55.9)	93
Allergic comorbidities, n (%)	43 (57.3)	75
Asthma, n (%)	15 (22.4)	67
Rhinitis, n (%)	30 (43.5)	69
Food allergy, n (%)	4 (9.1)	44
Cardiovascular comorbidities, n (%)	18 (23.4)	77
Beekeeper, n (%)	40 (43.0)	93
Clinical Data – index-reaction		
Age at reaction, M (IQR)	36 (26-48)	68
Severity classification (Müeller), M (IQR)	3 (2-3)	85
Grade 1, n (%)	12 (14.1)	85
Grade 2, n (%)	20 (23.5)	85
Grade 3, n (%)	35 (41.2)	85
Grade 4, n (%)	18 (21.2)	85
Number of stings in the same reaction, n (%)		
Once, n (%)	30 (75.0)	40
Twice, n (%)	4 (10.0)	40
Three or more times, n (%)	6 (15.0)	40
Clinical data – specific immunotherapy		
Patients undergoing VIT, n (%)	47 (50.5)	93
Completed treatment (median: 5 years), n (%)	19 (40.4)	47
Discontinued treatment, n (%)	2 (4.2)	47
Under treatment, n (%)	26 (55.4)	47
Adverse reactions during VIT, n (%)	16 (35.6)	45
Re-stung patients, n (%)	25 (53.2)	47
Systemic reactions, n (%)	5 (19.2)	26
Severity classification (Müeller)	2 (1-2)	5
Laboratory data		
Basal tryptase	4.2 (3.4-5.6)	72
Basal tryptase >11.4 ug/L, n (%)	3 (4.2)	72
Apis melífera IgE (total)	6.7 (1.0-20.3)	93
Apis mellifera IgE (VIT: pre-treatment)	13.7 (5.3-43.5)	47
Apis mellifera IgE (VIT: >12M treatment)	3.3 (1.1-7.9)	42
Api m 1 IgE	0.5 (0.1-1.6)	73
Positive (>0.34 kU/L), n (%)	42 (57.5)	73
Api m 4 IgE	0.01 (0.0-0.08)	42
Positive (>0.34 kU/L), n (%)	5 (11.9)	42
Api m 10 IgE	0.3 (0.1-1.6)	64
Positive (>0.34 kU/L), n (%)	32 (50.0)	64
Api m 1 (+) / 4 (-) / 10 (-), n (%)	5 (11.9)	42
Api m 1 (-) / 4 (+) / 10 (-), n (%)	0 (0.0)	42
Api m 1 (-) / 4 (-) / 10 (+), n (%)	2 (4.8)	42
Polysensitized *Api m 1/4/10 (+)*, n (%)	3 (7.1)	42

Legend: IQR – interquartile range; M – median.

Table II: Associations between the variables analyzed and the severity of the index-reaction (n=85)

Variables	Reaction severity grading (Müeller)				Total patients	*p-value*	Spearman Coefficient
	1 (n=12)	2 (n=20)	3 (n=35)	4 (n=18)			
Male gender, n (%)	7/12 (58.3)	11/20 (55.0)	21/35 (60.0)	9/18 (50.0)	85	*0.782*	
Age at time of index-reaction, M (IQR)	28.0 (12.0-40.0)	35.5 (27.0-45.5)	40.0 (29.0-53.0)	40.5 (28.5-55.0)	68	***0.040***	0.249
Allergic comorbidities, n (%)	5/11 (45.4)	7/13 (53.8)	18/30 (60.0)	11/14 (78.6)	68	*0.090*	
Cardiovascular comorbidities, n (%)	1/11 (9.1)	4/18 (22.2)	8/28 (28.6)	4/15 (26.7)	72	*0.308*	
Beekeepers, n (%)	5/12 (41.7)	7/20 (35.0)	16/35 (45.7)	7/18 (38.9)	85	*0.849*	
Basal tryptase, M (IQR)	5.2 (3.6-5.9)	3.7 (2.8-4.4)	4.2 (3.4-5.7)	5.0 (4.0-5.6)	64	*0.179*	0.170
Apis mellifera IgE (kU/L), M (IQR)	6.4 (0.01-12.4)	5.3 (1.1-10.6)	10.6 (1.9-42.8)	2.8 (0.4-34.7)	83	*0.409*	0.092
Api m 1 IgE (kU/L), M (IQR)	0.3 (0.02-1.3)	0.5 (0.2-1.3)	0.5 (0.1-1.6)	0.5 (0.01-2.1)	70	*0.804*	0.030
Positive (>0.34 kU/L), n (%)	5/11 (45.4)	12/17 (70.6)	14/25 (56.0)	9/17 (52.9)	70	*0.856*	
Api m 4 IgE (kU/L), M (IQR)	0.39 (0.01-0.77)	0.01 (0.01-0.02)	0.0 (0.0-0.04)	0.04 (0.01-0.18)	41	*0.734*	0.055
Positive (>0.34 kU/L), n (%)	1/2 (50.0)	1/10 (10.0)	1/17 (5.9)	2/12 (16.7)	41	*0.869*	
Api m 10 IgE (kU/L), M (IQR)	1.06 (0.23-1.31)	0.24 (0.07-0.44)	0.41 (0.11-1.99)	0.14 (0.02-2.59)	62	*0.580*	-0.072
Positive (>0.34 kU/L), n (%)	4/6 (66.7)	7/16 (43.8)	13/25 (52.0)	7/15 (46.7)	62	*0.765*	
Api m 1 (+) / 4 (-) / 10 (-), n (%)	1/2 (50.0)	3/10 (30.0)	1/17 (5.9)	0/12 (0.0)	41	***0.015***	
Api m 1 (-) / 4 (+) / 10 (-), n (%)	NA	NA	NA	NA	41	*NA*	
Api m 1 (-) / 4 (-) / 10 (+), n (%)	0/2 (0.0)	1/10 (10.0)	1/17 (5.9)	0/12 (0.0)	41	*0.658*	
Polysensitized *Api m 1/4/10 (+)*, n (%)	1/2 (50.0)	0/10 (0.0)	1/17 (5.9)	1/12 (8.3)	41	*0.736*	

Legend: M – median; IQR – interquartile range

Table III: Associations between the variables analyzed and the proportion of patients with reactions during VIT (n=45)

Variables	Reactions during VIT (n=45)		Total patients	*p-value*
	Yes (n=16)	No (n=29)		
Male gender, n (%)	10/16 (62.5)	20/29 (69.0)	45	*0.660*
Age at time of most severe reaction, M (IQR)	38 (31-42)	30 (21-37)	25	*0.113*
Severity of most severe reaction, M (IQR)	3 (3-4)	3 (2-3)	37	*0.142*
Allergic comorbidities, n (%)	6/13 (46.2)	9/25 (36.0)	38	*0.544*
Cardiovascular comorbidities, n (%)	1/14 (7.1)	1/19 (5.3)	33	*0.999*
Beekeeper, n (%)	11/16 (68.8)	16/29 (55.2)	45	*0.373*
Basal tryptase, M (IQR)	4.4 (3.1-5.0)	4.3 (3.5-5.7)	37	*0.340*
Apis mellifera IgE (pre-treatment), M (IQR)	16.4 (6.4-32.2)	12.4 (3.3-50.1)	45	*0.847*
Apis mellifera IgE (>12M treatment), M (IQR)	2.6 (1.1-8.0)	3.8 (0.6-6.7)	37	*0.808*
Api m 1 IgE (kU/L), M (IQR)	0.6 (0.4-1.0)	1.1 (0.3-2.9)	25	*0.397*
Positive (>0.34 kU/L), n (%)	8/10 (80.0)	10/15 (66.7)	25	*0.550*
Api m 4 IgE (kU/L), M (IQR)	0.04 (0.01-0.10)	0.01 (0.0-0.14)	19	*0.350*
Positive (>0.34 kU/L), n (%)	1/8 (12.5)	1/11 (9.1)	19	*0.999*
Api m 10 IgE (kU/L), M (IQR)	1.4 (0.5-2.6)	0.3 (0.2-2.9)	23	*0.418*
Positive (>0.34 kU/L), n (%)	9/10 (90.0)	6/13 (46.2)	23	***0.038***
Api m 1 (+) / 4 (-) / 10 (-), n (%)	0/8 (0.0)	2/9 (18.2)	19	*0.485*
Api m 1 (-) / 4 (+) / 10 (-), n (%)	NA	NA	NA	*NA*
Api m 1 (-) / 4 (-) / 10 (+), n (%)	1/8 (12.5)	0/11 (0.0)	19	*0.421*
Polysensitized *Api m 1/4/10 (+)*, n (%)	1/8 (12.5)	1/11 (9.1)	19	*0.999*

Legend: IQR – interquartile range; M – median.

Table IV: Associations between the variables analyzed and the proportion of patients with systemic reactions to re-stings (n=25)

Variables	Systemic reactions to re-stings (n=25)		Total patients	*p-value*
	Yes (n=4)	No (n=21)		
Male gender, n (%)	2/4 (50.0)	15/21 (71.4)	25	*0.660*
Age at time of most severe reaction, M (IQR)	28 (27-29)	36 (29-42)	11	*0.158*
Severity of most severe reaction, M (IQR)	3 (3-4)	3 (2-3)	21	*0.142*
Allergic comorbidities, n (%)	1/1 (100.0)	4/16 (25.0)	17	*0.100*
Cardiovascular comorbidities, n (%)	0/3 (0.0)	1/13 (7.7)	16	*0.999*
Beekeeper, n (%)	3/4 (75.0)	15/21 (71.4)	25	*0.999*
Basal tryptase, M (IQR)	3.0 (2.05-3.7)	4.25 (3.6-4.8)	22	*0.055*
Apis mellifera IgE (pre-treatment), M (IQR)	6.18 (3.3-37.61)	13.7 (5.89-41.2)	25	*0.543*
Apis mellifera IgE (>12M treatment), M (IQR)	4.28 (2.82-71.4)	1.97 (0.61-8.32)	23	*0.268*
Api m 1 IgE (kU/L), M (IQR)	1.02 (0.39-22.34)	0.54 (0.42-1.18)	12	*0.397*
Positive (>0.34 kU/L), n (%)	7/8 (87.5)	3/4 (75.0)	12	*0.999*
Api m 4 IgE (kU/L), M (IQR)	1.66 (0.00-3.14)	0.03 (0.01-0.14)	9	*0.350*
Positive (>0.34 kU/L), n (%)	2/3 (66.7)	0/6 (0.00)	9	*0.087*
Api m 10 IgE (kU/L), M (IQR)	0.15 (0.14-0.17)	1.08 (0.37-2.63)	11	*0.059*
Positive (>0.34 kU/L), n (%)	1/4 (25.0)	6/7 (85.7)	11	*0.093*
Api m 1 (+) / 4 (-) / 10 (-), n (%)	0/3 (0.0)	2/6 (33.3)	9	*0.500*
Api m 1 (-) / 4 (+) / 10 (-), n (%)	NA	NA	NA	*NA*
Api m 1 (-) / 4 (-) / 10 (+), n (%)	1/6 (16.7)	0/3 (0.0)	9	*0.999*
Polysensitized *Api m 1/4/10 (+)*, n (%)	2/3 (66.7)	0/6 (0.00)	9	*0.087*