ORIGINAL ARTICLE

CAP inhibition facilitates the identification of the primary sensitizing venom in patients with clinically presumed *Vespa crabro* stings: implications for venom immunotherapy selection

CAP inhibition in clinically presumed Vespa crabro allergy

Matteo Furlani[1], Daniela Visentini[2], Moira Busa[3], Alessandro Scarpa[4], Franco Borghesan[5], Francesca Rizzo[6], Valerio Pravettoni[7], Martina Fabris[1,2], Danilo Villalta[8]

[1]Department of Medicine, University of Udine, Udine, Italy

[2]Clinical Pathology Institute, Department of Laboratory Medicine, Friuli Centrale University Health Authority, Udine, Italy

[3]Allergy Unit, Mirano-Dolo, Venice, Italy

[4]Dermatology Unit, Mirano, Venice, Italy

[5]Allergy Unit, Department of Medicine, Cà Foncello Hospital, Treviso, Italy

[6]Allergy Unit, Department of Medicine, dell'Angelo Hospital, Mestre, Venice, Italy

[7]Department of Internal Medicine – Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

[8] Laboratory Immunology and Allergy Unit, Santa Maria degli Angeli Hospital, Pordenone, Italy

Summary

Background. Venom immunotherapy (VIT) is the only disease-modifying treatment for Hymenoptera venom allergy, and its effectiveness relies on accurate identification of the venom responsible for primary sensitization. In patients with clinically presumed *Vespa crabro* stings, this is challenging because extensive IgE cross-reactivity among Vespid venoms and limitations of current diagnostic reagents complicate interpretation of routine investigations. In this setting, CAP inhibition may provide complementary functional information to identify the primary sensitizing venom. **Methods.** We retrospectively evaluated 42 patients with systemic allergic reactions following clinically presumed *Vespa crabro* stings. The diagnostic work-up included clinical history, skin testing, serum-specific IgE to whole venom extracts, component-resolved diagnostics, and CAP inhibition. CAP inhibition results were integrated with clinical and laboratory findings to identify the primary sensitizing venom and support VIT selection. **Results.** Routine investigations frequently showed sensitization to multiple

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Vespid venoms, making identification of the primary sensitizing venom uncertain. Only one patient displayed a predominant *Vespa crabro*-specific IgE response, confirmed by CAP inhibition. Five additional patients had *Vespa crabro*-specific IgE levels comparable to those for other Vespids, together with elevated Api m 5-specific IgE, suggesting sensitization profiles influenced by highly cross-reactive homologous allergens. CAP inhibition improved diagnostic confidence by distinguishing primary sensitization (61.9%) from cross-reactive IgE responses and supported identification of the most appropriate venom for VIT. **Conclusions.** In clinically presumed *Vespa crabro* stings, the presumed culprit insect does not necessarily correspond to the primary sensitizing venom. Under current diagnostic conditions, CAP inhibition provides complementary functional information that improves identification of the primary sensitizing venom and may support more suitable VIT selection.

KEY WORDS

Vespa crabro; Hymenoptera venom allergy; venom immunotherapy; CAP inhibition; primary sensitizing venom; cross-reactivity; component-resolved diagnostics.

IMPACT STATEMENT

CAP inhibition could represent a potential contribution to the identification of the primary sensitizing venom in clinically presumed *Vespa crabro* allergic patients, distinguishing cross-reactivity from true sensitization and supporting more accurate VIT selection.

INTRODUCTION

Venom immunotherapy (VIT) is the only disease-modifying treatment for Hymenoptera venom allergy and provides highly effective protection against subsequent systemic reactions. Because VIT is venom-specific, accurate identification of the venom responsible for primary sensitization is the cornerstone of appropriate therapeutic selection (1–4). Consequently, the primary objective of the diagnostic work-up is not merely to document venom sensitization, but to identify the venom that should be prescribed for VIT. In some cases, multiple skin-test and/or serum IgE positivities may reflect cross-reactivity among components of different Hymenoptera venoms, such that VIT with a single venom may be sufficient. Conversely, unnecessary use of multiple venom extracts may increase treatment costs and potentially expose patients to a higher risk of adverse events or sensitization to additional allergens.

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In routine clinical practice, the diagnostic pathway usually begins with reconstruction of the sting event based on the patient's description of the insect responsible for the reaction. Although clinical history often provides a reliable indication of the culprit insect, its identification remains inherently presumptive, as it is based primarily on the patient's recollection of the insect's morphology and the circumstances of the sting. Therefore, identification of the presumed culprit insect should not automatically be considered equivalent to identification of the primary sensitizing venom, as they may diverge when extensive IgE cross-reactivity among Hymenoptera venoms is present (2,5–10).

This diagnostic challenge is particularly evident in patients with clinically presumed *Vespa crabro* stings. The venom of the European hornet contains homologous allergens shared with other Vespids, including Antigen 5, Phospholipase A1 and Hyaluronidase. The high structural identity of these homologous allergens accounts for the extensive IgE cross-reactivity commonly observed between *Vespa crabro, Vespula spp.* and, to a lesser extent, *Polistes dominula* (9). As a consequence, positive serum-specific IgE results to multiple Vespid venoms are frequently observed and may not necessarily reflect true multiple primary sensitizations.

The interpretation of routine diagnostic investigations is further complicated by the intrinsic limitations of the currently available *Vespa crabro* diagnostic reagents. Unlike the commercially available diagnostic extracts for *Vespula spp.* and *Polistes dominula*, which have been supplemented with additional Antigen 5 to improve diagnostic sensitivity, the currently available *Vespa crabro* diagnostic venom extract has not undergone similar optimization (11). Consequently, serum-specific IgE concentrations to *Vespa crabro* may be lower than those measured for *Vespula* or *Polistes,* even in patients in whom *Vespa crabro* is the clinically presumed culprit insect. Furthermore, species-specific molecular allergens for *Vespa crabro* are not yet available for CRD. Under these circumstances, identification of the primary sensitizing venom may remain uncertain despite a comprehensive conventional diagnostic work-up.

CRD significantly improved the evaluation of Hymenoptera venom allergy (7–10,12–18), but could not completely resolve the diagnosis in complex cases, particularly when sensitization involves homologous allergens displaying high structural identity across different venoms. In these selected patients, functional assays such as CAP inhibition may provide complementary information by assessing the relative contribution of individual venoms to the overall IgE response (12–13,19–20).

The present study evaluated the contribution of CAP inhibition within an integrated diagnostic approach in patients with clinically presumed *Vespa crabro* stings. The primary objective was to

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determine whether CAP inhibition could improve identification of the primary sensitizing venom when conventional diagnostic investigations remained inconclusive, thereby supporting identification of the most suitable VIT.

MATERIALS AND METHODS

Study design and patients

This retrospective observational study included consecutive patients referred to the Allergy Laboratory of Clinical Pathology Institute of the University Hospital of Udine from several allergy centers across the Friuli Venezia Giulia and Veneto regions for the evaluation of Hymenoptera venom allergy following a systemic allergic reaction to a clinically suspected *Vespa crabro* sting. Patients underwent a standardized allergy evaluation conducted by experienced allergists. Because entomological confirmation was only occasionally available, identification of the presumed culprit insect was primarily based on a detailed clinical history, including the patient's description of the insect, its body size and morphology, flight behaviour, the circumstances and location of the sting, the environmental setting, seasonality, and, when available, nest characteristics. Additionally, patients were shown photographs of different Hymenoptera species to aid species identification. As in routine clinical practice, insect identification was considered clinically presumptive and served as the starting point for the diagnostic work-up.

Patients were included if they had experienced a systemic reaction, classified according to Mueller's classification, compatible with an IgE-mediated hypersensitivity reaction and had completed the entire diagnostic protocol. Patients with incomplete diagnostic investigations or insufficient clinical information were excluded.

Forty-two consecutive patients fulfilled the inclusion criteria. Thirty-nine experienced severe systemic reactions (Mueller grades III–IV), whereas three experienced grade II reactions.

The study was conducted according to the principles of the Declaration of Helsinki and written informed consent was obtained from all patients.

Diagnostic work-up

All patients underwent a standardized stepwise diagnostic evaluation including detailed clinical history; skin testing with Hymenoptera venom extracts; serum-specific IgE to whole venom extracts;

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CRD; and CAP inhibition assay with *Vespula spp., Vespa crabro* and *Polistes dominula* venoms. All tests were conducted between 1 and 6 months after the sting.

Skin tests

Skin prick tests and intradermal tests were performed using commercially available venom extracts (Anallergo, Florence, Italy) according to current international recommendations. Test concentrations and positivity criteria followed the manufacturer's instructions and contemporary EAACI recommendations.

Serum-specific IgE and tryptase

Routine laboratory evaluation included serum-specific IgE against whole venom extracts of *Vespa crabro* (i75), *Vespula spp.* (i3) and *Polistes dominula* (i77) using the ImmunoCAP system (Thermo Fisher Scientific, Uppsala, Sweden). CRD included Api m 1 (phospholipase A2), Api m 5 (dipeptidyl-peptidase IV), Ves v 1 (phospholipase A1), Ves v 5 (Antigen 5), Pol d 5 (Antigen 5), and cross-reactive carbohydrate determinants (CCD; MUXF3), measured by ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden). Results were expressed as kUA/L, and values ≥0.10 kUA/L were considered positive. Baseline serum tryptase was also measured by ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden).

CAP inhibition assay

CAP inhibition was performed according to the protocol of Straumann et al. (21). The assay was performed in sera with venom-specific IgE levels >1 kUA/L. Briefly, patient sera were pre-incubated overnight at 4–8 °C with venom extracts of *Vespula spp., Polistes dominula* and *Vespa crabro* (Anallergo, Florence, Italy) at concentrations of 300, 30, 3, and 0.3 µg/mL; residual venom-specific IgE levels were then determined using the ImmunoCAP method.

Inhibition results were interpreted by evaluating the overall dose–response profiles across the entire range of inhibitor concentrations, rather than relying on inhibition values obtained at a single concentration. Homologous inhibition exceeding 80% at the highest inhibitor concentration (300 µg/mL) was considered indicative of adequate assay performance. Heterologous inhibition was assessed by evaluating the ability of each venom to inhibit IgE binding to the other venom, considering both the magnitude of inhibition and the corresponding dose–response profiles. A marked asymmetry in heterologous inhibition profiles was considered suggestive of a predominant sensitization to the venom exhibiting the stronger inhibitory capacity, whereas comparable bidirectional inhibition profiles

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were interpreted as indicative of extensive IgE cross-reactivity. Dual primary sensitization was considered when both venoms exhibited substantial homologous inhibition but limited heterologous inhibition in either direction. No predefined percentage cut-off was applied to heterologous inhibition, which was interpreted on the basis of the overall inhibition profiles.

Statistical analysis

Continuous variables are presented as median (interquartile range), whereas categorical variables are reported as absolute numbers and percentages. Differences among paired venom-specific IgE concentrations were analysed using the Friedman test followed by Wilcoxon signed-rank tests with Bonferroni correction. A two-sided P value <0.05 was considered statistically significant.

RESULTS

Diagnostic findings

Routine in vivo and in vitro diagnostic investigations frequently revealed multiple sensitizations to Vespid venoms. Serum-specific IgE positivity to both *Vespula spp*. and *Polistes* was detected in all 42 patients, while sensitization to *Vespa crabro* was observed in 41 of the 42 patients. The positivity rates for Ves v 1, Ves v 5, Pol d 5, Api m 5, and MUXF3 (CCD) are reported in **Table I.** Consequently, routine diagnostic investigations alone rarely enabled the confident identification of the primary sensitizing venom.

Whole-venom extract-specific IgE concentrations differed significantly among the three investigated venom extracts **(Figure 2**). Median *Vespa crabro*-specific IgE concentrations were significantly lower than those observed for *Vespula spp*. and *Polistes dominula* (Friedman test, P<0.001; post-hoc Wilcoxon tests, both P<0.0001). Only one patient (1/42, 2.4%) showed a *Vespa crabro*-specific IgE concentration exceeding those to both *Vespula spp*. and Polistes by more than 50%, suggesting primary sensitization to *Vespa crabro*, confirmed by CAP inhibition assay. Five additional patients (5/42, 11.9%) showed *Vespa crabro*-specific IgE concentrations comparable to those observed for *Vespula* and/or *Polistes.* All five patients also presented elevated Api m 5-specific IgE. These data confirm that quantitative extract-specific IgE values were insufficient to identify the venom most suitable for therapeutic decision-making.

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CAP-inhibition assessment

CAP inhibition identified seven diagnostic patterns (**Table II**). The most frequent pattern consisted of combined equal sensitization to *Vespula spp.* and *Vespa crabro* (13/42, 30.9%), followed by isolated *Vespa crabro* sensitization (6/42, 14.3%) and dual *Vespula–Polistes* sensitization (6/42, 14.3%). Isolated *Vespula spp.* sensitization was identified in 5 patients (11.9%), while isolated *Polistes* sensitization and dual *Polistes–Vespa* crabro sensitization were each observed in 5 patients (11.9%). Triple sensitization involving *Vespula, Polistes* and *Vespa crabro* with comparable inhibition was identified in two patients (4.8%). *Vespa crabro* venom, either alone or in association with other venoms, was identified as a potential primary sensitizing agent in 26 of 42 (61.9%) evaluated patients. These findings demonstrate the marked heterogeneity of sensitization profiles among patients referred after clinically presumed *Vespa crabro* stings.

DISCUSSION

The present study demonstrates that, in patients with clinically presumed *Vespa crabro* stings, identification of the presumed culprit insect does not necessarily correspond to identification of the clinically relevant sensitizing venom. Under current diagnostic conditions, CAP inhibition provided complementary functional information that increased diagnostic confidence in patients whose sensitization profiles remained difficult to interpret after routine investigations. Rather than replacing conventional diagnostic tools, CAP inhibition contributed to an integrated diagnostic assessment aimed at supporting selection of the most suitable venom for VIT (1–4,10,12–13).

The distinction between the presumed culprit insect and the primary sensitizing venom represents the central concept emerging from our findings. In routine clinical practice, the diagnostic evaluation of Hymenoptera venom allergy inevitably begins with reconstruction of the sting event. The patient's description of the insect, together with the clinical circumstances of exposure, often provides a reliable indication of the likely culprit insect. Nevertheless, this identification remains inherently presumptive and should not automatically be considered equivalent to identification of the primary sensitizing venom. These two diagnostic objectives frequently coincide, but they may diverge in patients showing extensive IgE cross-reactivity between different Vespid venoms. Accordingly, the presumed culprit insect represents the starting point of the diagnostic pathway, whereas identification of the primary sensitizing venom represents its therapeutic endpoint because it ultimately guides VIT selection (18–20).

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A very important factor in identifying the primary sensitizing Hymenoptera species is the patient's previous history of stings. If the patient has previously been stung by *Vespula spp.* but has never been stung by *Vespa crabro, Vespula spp*. can reasonably be considered the primary sensitizing species. In such cases, the systemic reaction following a subsequent *Vespa crabro* sting may be explained by the fact that *Vespa crabro* typically injects a larger amount of venom than *Vespula spp.* Conversely, if the patient has previously experienced stings from both *Vespula spp.* and *Vespa crabro,* a true double sensitization should be considered. Under these circumstances, the CAP inhibition assay is particularly valuable, as it enables discrimination between antigenic cross-reactivity and genuine species-specific IgE sensitization to venom proteins from either *Vespula spp.* or *Vespa crabro.*

This diagnostic challenge is particularly relevant in patients with clinically presumed *Vespa crabro* stings. Unlike the commercially available diagnostic extracts for *Vespula spp.* and *Polistes dominula,* which have been supplemented with additional Antigen 5 to improve diagnostic sensitivity, the currently available *Vespa crabro* extract has not undergone similar optimization (11). Moreover, species-specific molecular allergens for *Vespa crabro* are not yet available for routine clinical use. Consequently, serum-specific IgE concentrations to *Vespa crabro* are frequently lower than those observed for *Vespula* or *Polistes*, even when *Vespa crabro* represents the clinically presumed culprit insect. Under these circumstances, conventional diagnostic investigations may be insufficient to identify the primary sensitizing venom with adequate confidence.

The frequent coexistence of positive IgE responses to *Vespula and Vespa crabro* should not automatically be interpreted as evidence of double primary sensitization. These venoms contain homologous allergens, including Antigen 5, Phospholipase A1, Hyaluronidase and Dipeptidyl-peptidase, which share a high structural identity and account for the extensive IgE cross-reactivity commonly observed in clinical practice. From a therapeutic perspective, this distinction is important because combined IgE reactivity to *Vespula and Vespa crabro* is more likely to reflect cross-reactivity than genuine double-primary sensitization. Conversely, identification of patients in whom *Vespa crabro* is not the clinically relevant sensitizing venom—particularly when the integrated diagnostic assessment is more consistent with sensitization to *Polistes dominula*—may have greater relevance for VIT selection (**Table III**).

Particular attention should be paid to the subgroup of patients showing comparable *Vespa crabro*-specific IgE concentrations together with elevated Api m 5-specific IgE. Api m 5 is a highly cross-reactive dipeptidyl peptidase present in the venoms of several Hymenoptera species (22). Although the present study was not designed to investigate the diagnostic performance of Api m 5 itself, these

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findings suggest that sensitization to highly conserved homologous allergens may contribute to complex IgE profiles in patients with clinically presumed *Vespa crabro* stings. Indirectly, these observations also support the hypothesis that further optimization of the *Vespa crabro* diagnostic extract, including additional supplementation with Antigen 5, may improve the diagnostic performance of routine extract-specific IgE assays.

The present findings should not be interpreted as suggesting that CAP inhibition should replace conventional diagnostic investigations or become a first-line diagnostic test. On the contrary, our results support its role as a complementary functional assay that may be particularly useful in carefully selected patients whose routine diagnostic work-up remains inconclusive. CAP inhibition should therefore be interpreted together with clinical history, skin testing, extract-specific IgE and CRD within an integrated diagnostic assessment. Its principal contribution is not to establish the diagnosis in isolation, but to increase diagnostic confidence when selecting the appropriate venom for VIT.

Our study has several limitations. Its retrospective design and the relatively small sample size reflect the rarity of clinically well-characterized *Vespa crabro* sting reactions evaluated by CAP inhibition. In addition, CAP inhibition is currently available only in specialized laboratories and requires specific technical expertise. Detailed information on the number of previous Hymenoptera stings and on the species responsible for those stings was not systematically available for individual patients. Therefore, we could not assess the potential influence of prior sting exposure on sensitization patterns and CAP-inhibition results, and this should be considered when interpreting the inferred primary sensitization. The present study was not designed to assess the clinical effectiveness of VIT based on the integrated diagnostic classification proposed here. Therefore, the potential impact of this diagnostic approach on long-term clinical protection should be evaluated in prospective studies. Another limitation is the lack of follow-up data confirming the appropriateness of the selected venom for VIT. None of the patients experienced a field sting during follow-up, and sting challenge is not permitted in Italy, despite the availability of European guidelines supporting its use.

In conclusion, our findings support an integrated venom-centred diagnostic approach in patients with clinically presumed *Vespa crabro* stings. Identification of the primary sensitizing venom, rather than reliance on the presumed culprit insect alone, represents the key step for selecting the most appropriate VIT. Within this integrated diagnostic pathway, CAP inhibition may provide complementary functional information that may substantially increase diagnostic confidence in carefully selected diagnostically challenging patients.

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FUNDING

This research received no external funding.

INSTITUTIONAL REVIEW BOARD STATEMENT

The study was conducted in accordance with the principles of the Declaration of Helsinki.

INFORMED CONSENT STATEMENT

Written informed consent was obtained from all participants, according to institutional requirements.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding author upon reasonable request. Data are not publicly available because they contain information that could compromise participant privacy.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Figure 1. Representative CAP inhibition curves illustrating: (A) co-primary sensitization to Vespa crabro and Vespula spp.; (B) primary sensitization to Vespula spp. For inhibition with Polistes dominula venom, only the value measured at 300 µg/mL is shown. Solid lines indicate homologous inhibition; dashed lines indicate heterologous inhibition.

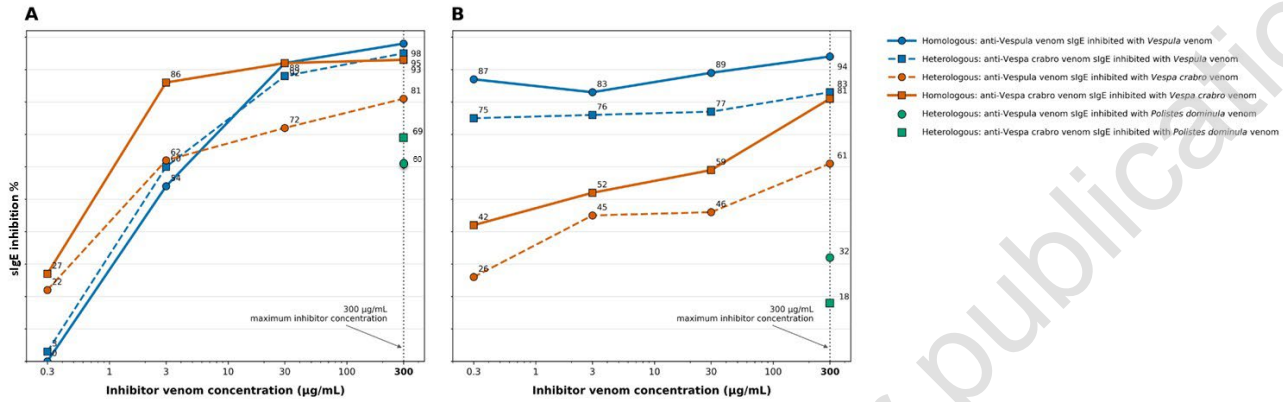
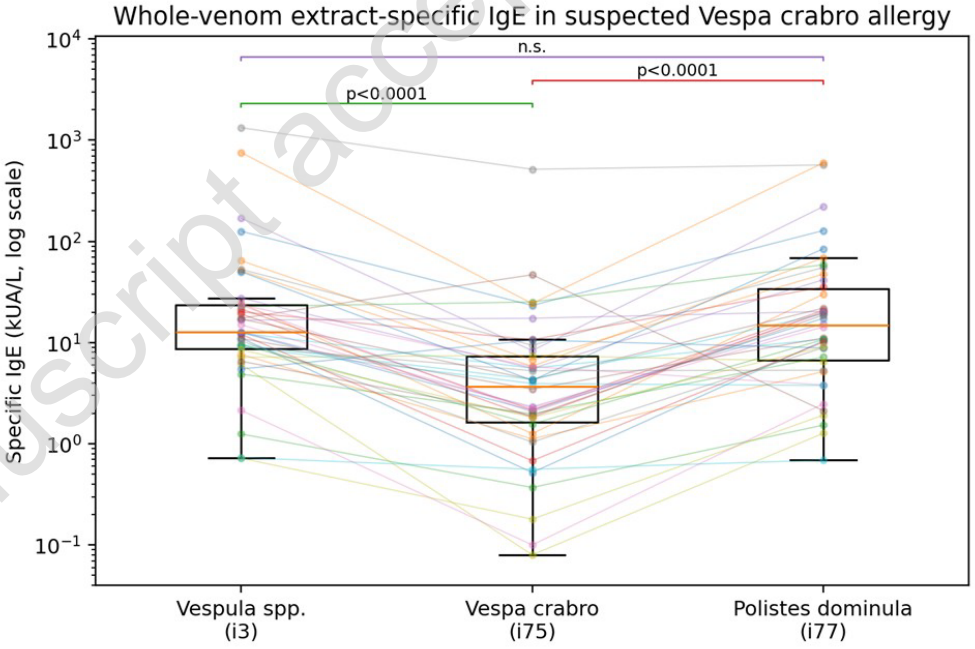


Figure 2. Distribution of specific IgE to whole-venom extracts. Individual paired values are shown on a logarithmic scale. Vespa crabro extract-specific IgE (i75) was significantly lower than both Vespula spp. (i3) and Polistes dominula (i77), supporting a likely analytical limitation of the i75 extract rather than a true absence of Vespa crabro sensitization.



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TABLE I

Baseline demographic, clinical and laboratory characteristics of the study population

Variable	Value
Patients, n	42
Age: years mean ± SD (range)	58.3 ± 17.3 (16–84)
Male/Female sex, n	30/12
Clinical history suggestive of Vespa crabro sting, n (%)	42 (100%)
Mueller grade III–IV, n (%)	39 (92.9%)
Baseline serum tryptase, µg/L, median (IQR)	6.0 (4.3–6.6)
Positive sIgE to Apis mellifera (i1), n/N (%)	27/42 (64.3%)
Positive sIgE to Vespula spp. (i3), n/N (%)	42/42 (100.0%)
Positive sIgE to Vespa crabro (i75), n/N (%)	41/42 (97.6%)
Positive sIgE to Polistes dominula (i77), n/N (%)	42/42 (100.0%)
Positive Ves v 5, n/N (%)	42/42 (100.0%)
Positive Pol d 5, n/N (%)	42/42 (100.0%)
Positive Ves v 1, n/N (%)	28/42 (66.7%)
Positive Api m 5, n/N (%)	25/38 (65.8%)
Positive CCD (MUXF3), n/N (%)	14/42 (33.3%)

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TABLE II

Distribution of the seven diagnostic patterns identified by CAP inhibition assay.

Diagnostic assessment	Patients n (%)
Vespula spp.–Vespa crabro (equal inhibition)	13 (30.9%)
Vespula spp.	5 (11.9%)
Vespula spp.–Polistes dominula (dual sensitization)	6 (14.3%)
Vespa crabro	6 (14.3%)
Polistes dominula–Vespa crabro (dual sensitization)	5 (11.9%)
Polistes dominula	5 (11.9%)
Vespula spp.–Polistes dominula–Vespa crabro (equal inhibition)	2 (4.8%)

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TABLE III

Hypothesis-generation framework for integrating CAP inhibition findings into the overall diagnostic assessment supporting VIT selection in patients with clinically presumed Vespa crabro stings.

Integrated diagnostic interpretation	Potential contribution to VIT selection
Evidence consistent with primary Vespa crabro sensitization	Supports consideration of Vespa crabro venom for immunotherapy
Evidence consistent with primary Vespula spp. sensitization	Supports consideration of Vespula spp. venom for immunotherapy
Evidence consistent with primary Polistes dominula sensitization	Supports consideration of Polistes venom for immunotherapy
Predominantly cross-reactive Vespula spp.–Vespa crabro sensitization profile	Individualized selection based on the overall integrated diagnostic assessment
Evidence consistent with dual primary sensitization: Vespula spp. and Polistes dominula	Supports consideration of dual-venom immunotherapy: Polistes dominula and Vespula spp.
Evidence consistent with dual primary sensitization: Polistes dominula and Vespa crabro	Supports consideration of dual-venom immunotherapy: Polistes dominula and Vespa crabro

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