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Wasp venom allergy screening with recombinant allergen testing. Diagnostic performance of rPol d 5 and rVes v 5 for differentiating sensitization to *Vespula* and *Polistes* subspecies

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ABSTRACT

Background: The appropriate therapeutic management of patients with wasp-induced allergic reactions necessitates an accurate allergologic workup, entailing differentiation between sensitization against *Vespula* or *Polistes* venoms.
Materials and methods: We studied 52 consecutive adult subjects diagnosed with hypersensitivity to wasp venoms. Cap inhibition was performed using UniCAP Specific IgE. The concentration of serum IgE against two recombinant constituents of Antigen 5 (rVes v 5 and rPol d 5) was also measured using Immuno CAP 250. The ratio between values of specific IgE against recombinant allergens was calculated and a percentage difference >50% was considered significant for specific immunization against one of the two venoms.
Results: The diagnostic agreement between recombinant allergens testing and CAP inhibition was 54% (kappa statistics, 0.34; 95% CI, 0.18–0.50) in the whole study population. In the 24 patients with recombinant allergens ratio >50% and non dubious results of CAP inhibition assay the diagnostic agreement was perfect (100%; kappa of agreement, 1.00; 95% CI; 1.00–1.00).
Discussion: The results of this study show that the assessment of specific IgE against rVes v 5 and rPol d 5 may be regarded as a low-cost screening, providing valuable diagnostic information for differentiating the sensitization against *Vespula* or *Polistes* venoms. In patients with suggestive clinical history and ratio >50% between specific IgE against rVes v 5 and specific IgE against and rPol d 5, the CAP inhibition assay may be safely withheld, thus allowing to achieve an early diagnosis at lower cost.

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1. Introduction

The current armamentarium of in vitro diagnostic testing for assessment of Hymenoptera hypersensitivity in the case of in vivo polysensitization entails a large number of highly purified allergens and/or peptides generated by molecular biology techniques [1]. Although the diagnostic of bee sensitization represents a peculiar challenge due to the fact that positivity to wasp IgE may be present in as many as 50% of patients [2], the introduction of recombinant (r) phospholipase A2 has represented a considerable advancement, leading to a considerable improvement of diagnostic specificity compared to the use of natural honey bee venom [3]. In particular, it has been convincingly demonstrated that the use of a mixture of the three major T-cell epitope of phospholipase A2 may substantially improve the diagnostic sensitivity with no significant decrease of diagnostic specificity [4]. Nevertheless, this approach is still plagued by a suboptimal diagnostic efficiency [4].

In serum samples exhibiting cross reactivity between bee and wasp venom, the presence of IgE against cross-reactive carbohydrate determinants (CCD) occurs in up to 80% of samples testing positive for both species [5]. Interestingly, in the Mediterranean area the diagnostic problem attributable to cross-reactivity not only emerges between bee and wasp venom, but also between venoms produced by different subspecies of wasps, so that the choice of the most appropriate immunotherapy is further challenged by the difficulty to distinguish which subspecies is responsible for the sting [6]. Since *Vespula germanica* and *Polistes dominulus* are similarly distributed in southern Europe, Italy, Spain and Croatia [7,8], distinguishing the cross reactivity between bees and wasps, as well as between different subspecies of wasps, represents an

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Table 1
Demographic and laboratory characteristics (median and interquartile range) of the study population.

n	52
Age (years)	48 (43–58)
Sex (women/Men)	10/42
Total IgE (kU/L)	98 (69–186)
Positivity to skin test (%)	
·*Polistes*	33/52 (63%)
·*Vespula*	18/52 (35%)
·Dubious	1/52 (2%)
Positivity to CAP inhibition (%)	
·*Polistes*	30/52 (58%)
·*Vespula*	16/52 (31%)
·Dubious	6/52 (11%)
rVes v 5 (kU/L)	2.68 (0.55–7.63)
rPol d 5 (kU/L)	3.03 (1.10–6.22)

essential part of the diagnostic workup. Importantly, diagnostic techniques such as CAP inhibition show great promise to enhance the diagnostic accuracy, and may also reduce the number of IgE inhibition assays, thus decreasing the overall expenditure [9]. Indeed, an efficient use of this promising diagnostic technique also requires better assessment of the cross reactivity between the wasp subspecies *Vespula* and *Polistes* [6]. Considerable evidence has accumulated from studies exploring the structural heterogeneity and diagnostic accuracy of recombinant phospholipase A1 and phospholipase A2, as well as on the clinical usefulness of CCD [6]. Nevertheless, there is scarce and even contradictory information about the use of Antigen 5 for differentiating among the different subspecies of wasps. Ves v 5 and Pol d 5, two main constituents of Antigen 5, have recently been identified as the most potent allergens of both *Vespula* spp. and *P. dominulus* venom, respectively, but they could not be detected in honey bee venom [10]. Therefore, this study was aimed to assess whether the assessment of rVes v 5 and rPol d 5 may generate clinically valuable information in the IgE response of patients with potential polysensitization to the wasp subspecies *Vespula* and *Polistes*.

2. Materials and methods

The study population consisted in 52 consecutive adult subjects who were diagnosed with cutaneous hypersensitivity to wasp venom and a clinical history of III and IV grade reaction [11]. Prick tests and intradermal tests to vespid venoms were performed according to the reference European Academy of Allergy and Clinical Immunology (EAACI) criteria [12], using commercial extracts (*Vespula* spp., Stallergènes, Milan, Italy; *P. dominulus* and *Vespa crabro*, Anallergo, Florence, Italy). Reactions equaling or exceeding a diameter of 3 mm after 15 min were considered positive. Blood samples were collected into evacuated blood tubes without additives upon admission to the Allergy unit of the University Hospital of Verona. Blood was separated by standard centrifugation at 1500 × *g* for 15 min, and the serum was stored at −30 °C until measurement. CAP inhibition was performed using UniCAP Specific IgE (Immuno CAP 250, Thermo Fisher Scientific, Uppsala, Sweden). The technical and analytical characteristics of this assay have been previously described elsewhere [9,13,14]. The linearity of the assay is comprised between 0 and 100 kU/L. Although serum values >0.1 kU/L are suggestive for sensitization, the diagnostic threshold was fixed at >0.35 kU/L in agreement with results obtained in a previous study [15]. In all samples the IgE pattern was further defined using ImmunoCAP® inhibition assay technique (Thermo Fisher Scientific), in which serum samples are pre-incubated with homologous or heterologous venoms before testing for venom-specific IgE antibodies. Briefly, a stock solution (300 g/mL) of both wasp and bee venoms was serially diluted to obtain scalar venom concentrations of 3 g/mL, 30 g/mL and 150 g/mL using a specific sample diluent (Thermo Fisher). Then, 100 μL of serum were incubated overnight at 4–8 °C with 200 μL of the five venom dilutions (i.e., from 0 to 300 g/mL), and specific IgE were assessed the following day with UniCAP Specific IgE (Thermo Fisher Scientific). Results were plotted against a calibration curve obtained with increasing concentration of specific IgEs (0, 0.35, 0.7, 3.5, 17.5 and 100 kU/L). Results were considered dubious for values lower than 75%, as reported elsewhere [16].

The concentration of serum IgE against rVes v 5 and rPol d 5 was measured using Immuno CAP 250 (Thermo Fisher Scientific). The ratio between values of specific IgE against rVes v 5 and rPol d 5 was calculated in all samples, and a percentage difference > 50% was considered significant. The positivity to either *Polistes* or *Vespula* with the ImmunoCAP® inhibition assay and the positivity to rVes v 5 or rPol d 5 were compared with kappa of agreement and its 95% confidence interval (95% CI), using Analyse.it (Analyse-it Software Ltd, Leeds, UK). All patients provided a written consent for being enrolled in this investigation. The study was cleared by the local Institutional Review Board, and was carried out in accord with the Declaration of Helsinki and under the terms of all relevant local legislation.

3. Results

The main results of this study are shown in Table 1. The skin test was positive for *Polistes* in 63% of cases, *Vespula* in 35% of cases, whereas results were classified as dubious in the remaining 2% of cases. The CAP inhibition test was positive for *Polistes* in 58% of cases, *Vespula* in 31% of cases, whereas results were classified as dubious in the remaining 11% of cases. The overall agreement between skin test and Cap inhibition was 90% (kappa statistics, 0.82; 95% CI, 0.68 to 0.96; $p < 0.001$).

As regards recombinant allergen testing, 26/52 patients (50%) displayed a ratio between rVes v 5 and rPol d 5 values >50% (8/52 with rVes v 5 higher than rPol d 5; 18/52 with rPol d 5 higher than rVes v 5), in 25/52 cases the ratio was ≤50%, whereas in the remaining case the values of IgE against both allergens were considered too low for calculating the ratio (i.e., 0.11 kU/L for rVes v 5 and undetectable for rPol d 5, respectively). In 4/52 patients (8%) the CAP inhibition test was dubious and the ratio between rVes v 5 and rPol d 5 values was <50%.

Overall the diagnostic agreement between recombinant allergens testing and CAP inhibition in the whole study population (i.e., including the "dubious" results of CAP inhibition testing and the

Diagnostic agreement between CAP inhibition and recombinant allergen testing the entire study population.

	CAP inhibition			
Recombinant allergens	Polistes	Undetermined	Vespula	Total
Polistes	17	1	0	18
Undetermined	13	4	9	26
Vespula	0	1	7	8
Total	30	6	16	52

Agreement: 54% (kappa statistics, 0.34; 95% CI, 0.18-0.50; p<0.001).

Fig. 1. Diagnostic agreement between CAP inhibition and recombinant allergen testing the entire study population. Agreement: 54% (kappa statistics, 0.34; 95% CI, 0.18–0.50; $p < 0.001$).

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