

Improved recombinant Api m 1- and Ves v 5-based IgE testing to dissect bee and yellow jacket allergy and their correlation with the severity of the sting reaction

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Summary

Background No study has assessed the diagnostic sensitivity of rApi m 1 and rVes v 5 on Immulite testing system.

Objective To compare the diagnostic sensitivity of commercially available venom recombinant allergens between the currently available immunoassays [ImmunoCAP (CAP) and Immulite (LITE)] and establish their correlation with the severity of the sting reaction.

Methods This study evaluated 95 bee venom and 110 yellow jacket venom-allergic subjects. We measured the levels of sIgE to rApi m 1, rVes v 5 (LITE and CAP), rApi m 2 (LITE), rVes v 1 (CAP) and total IgE (CAP). Forty-nine healthy subjects served as controls.

Results The diagnostic sensitivity of rApi m 1 and rVes v 5 was significantly higher with the LITE than with the CAP system (71% vs. 88% and 82% vs. 93%). The specificity of both assays for both allergens was between 94% and 98%. Twenty-nine patients that tested negative for rApi m 1 or rVes v 5 with CAP were positive with LITE, but none of the patients that tested negative with LITE were positive with CAP. The positive values of rApi m 1 and rVes v 5 were on average 2.7 and 2.3 times higher, with the LITE than with the CAP system. The combination of rApi m 1 and rApi m 2 (LITE) and the combination of rVes v 5 (LITE) and rVes v 1 (CAP) almost matched the sensitivity of native venoms (95% and 97%, respectively), whereas the diagnostic sensitivity of the combination of rVes v 5 and rVes v 1 (CAP) did not reach the sensitivity of rVes v 5 (LITE) alone (90% vs. 93%). IgE levels to venom recombinants and total IgE did not correlate with the severity of sting reaction.

Conclusions & Clinical Relevance The use of rApi m 1 and rVes v 5 with the LITE system significantly enhanced diagnostic utility of venom recombinants and should improve the dissection of bee and yellow jacket venom allergy.

Keywords rApi m 1, rVes v 5, ImmunoCAP, Immulite, enhanced sensitivity, comparison

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Introduction

The measurement of specific IgE antibodies (sIgE) against native Hymenoptera venom is valuable in diagnosing Hymenoptera venom allergy. However, if judgments on the source insect are based on the results of sIgE measurements, the proper identification of the offending Hymenoptera species is hindered because sIgE antibodies can be double positive (positive to the native bee and to native wasp venom) in up to 60% [1, 2] of patients, even though less than 10% [3] of patients that are allergic to Hymenoptera venom have a systemic

reaction after being stung by insects from both families (Vespidae spp. and Aptidae spp.). The false double positivity of sIgE measurements is primarily due to either antibodies against cross-reactive carbohydrate determinants (so-called CCD antibodies) that are present in venom from both families or antibodies against epitopes of homologous proteins of both venoms, such as hyaluronidase or dipeptidylpeptidase [4]. In the last decade, preparations with recombinant venom components that are devoid of CCDs have been used in routine clinical practice because they enable the discrimination between genuine double sensitization

and cross-reactivity [5]. There are currently two systems for the quantitative detection of recombinant venom IgE antibodies used in routine practice: the ImmunoCAP solid phase assay (Thermo Fisher Scientific, Waltham, MA, USA) and Immulite liquid allergen testing system (Siemens AG, Erlangen, Germany). In CAP system, the allergen is covalently bound to a solid phase consisting of an activated hydrophilic polymer. The secondary anti-IgE antibodies are bound to the enzyme B-galactosidase, which transforms added methylumbelliferyl-B-D-galactoside into a fluorescent product. The LITE system uses allergens bound covalently to soluble biotinylated polylysine polymers in a fluid phase that in turn binds to a streptavidin-covered polystyrene ball through a streptavidin-biotin complex. The secondary anti-IgE antibodies are conjugated to alkaline phosphatase acting on an adamantyl-dioxetane phosphate ester substrate to emit a chemiluminescent signal.

The first routinely used major honeybee venom recombinant allergen rApi m 1 (phospholipase A2; i208) became available for the CAP assay in 2009. It is a substitute for the previously used natural allergen Api m 1. The main limitation of this particular commercially available recombinant Api m 1 allergen is its low sensitivity (approximately 70%) [5–8]. This value is much lower than the sensitivities of native or custom recombinant Api m 1 allergen preparations, which have been reported to reach more than 90% [2, 9–11].

Because of the aforementioned limitations in the sensitivities of recombinant proteins in the CAP system, additional recombinant allergens (rApi m 2, rApi m 3, nApi m 4, rApi m 5, rApi m 10) have been produced to enhance sensitivity. However, these preparations are not yet commercially available [8].

The first routinely used recombinant allergen from yellow jacket (*Vespula vulgaris*) venom rVes v 5 (antigen 5; i209) became available in 2010 for the CAP system. It has higher sensitivity than commercially used rApi m 1, but it does not match the sensitivity level of the native venom [12]. Therefore, another recombinant allergen of yellow jacket venom, the rVes v 1 (phospholipase A1; i211) allergen, was produced for CAP. The combined usage of rVes v 5 and rVes v 1 had slightly increased sensitivity, but it still did not match the sensitivity of the native venom [12].

In 2013, routine recombinant Api m 1 (a45), Api m 2 [hyaluronidase, (a46)] and Ves v 5 (a670) allergen preparations also became available for the LITE system. To our knowledge, no study has assessed the sensitivities of these routinely used recombinant allergens or compared them to other commercially available recombinant allergens.

For this reason, we sought to assess the sensitivities of LITE's rApi m 1, rApi m 2 and rVes v 5 allergen

preparations and compare them to CAP's rApi m 1, rVes v 5 and rVes v 1 allergen preparations, thus exploring all of the recombinant honeybee and yellow jacket venom allergen preparations commercially available to date.

We also tested whether the results of measurements of any of the components of the IgE system (sIgE, total IgE and recombinant IgE) correlated with reaction severity, because some publications [13] have shown that higher total IgE levels are related to milder reaction severities, although we were not able to confirm this fact with our clinical observations. We speculated that levels of recombinant venom components may be linked to reaction severity, as they are devoid of CCD components and measure only the disease-relevant concentrations of IgE antibodies.

Methods

Patients

Patients allergic to bee and yellow jacket venom, with different reaction severities (please see below), were included in the study. We included a roughly equal number of patients allergic to bees ($n = 95$) and patients allergic to yellow jackets ($n = 110$).

Reaction severity

Patients were divided into three groups according to their reaction severity:

- Local reaction group (LR): This group consisted of 25 patients who had a large local reaction without any systemic signs after an insect sting.
- Systemic reaction group (SR): This group consisted of 70 patients who had a mild systemic reaction without any signs of cardiorespiratory involvement (Mueller grade 1 and 2) after an insect sting.
- Anaphylactic reaction group (AR): This group consisted of 110 patients who had a severe systemic reaction with signs of cardiorespiratory involvement (Mueller grade 3 and 4) after an insect sting.

Laboratory tests

All bee venom-allergic patients had positive measurements of IgE antibodies to bee venom (i1) and negative measurements to yellow jacket venom (i3). All yellow jacket venom-allergic patients had positive measurements of IgE antibodies to yellow jacket venom (i3) and negative measurements to bee venom (i1). The measurements of IgE antibodies against native Hymenoptera venoms were performed using CAP technique.

Depending on which venom the patient was allergic to, a battery of different diagnostic tests was performed:

- Patients allergic to bee venom:
 - Measurements of IgE against recombinant Api m 1 allergen with CAP (rApi m 1 CAP [i208]).
 - Measurements of IgE against recombinant Api m 1 allergen with LITE (rApi m 1 LITE [a45]).
 - Measurements of IgE against recombinant Api m 2 allergen with LITE (rApi m 2 LITE [a46]).
- Patients allergic to yellow jacket venom:
 - Measurements of IgE against recombinant Ves v 5 allergen with CAP (rVes v 5 CAP [i209]).
 - Measurements of IgE against recombinant Ves v 5 allergen with LITE (rVes v 5 LITE [a670]).
 - Measurements of IgE against recombinant Ves v 1 allergen with CAP (rVes v 1 CAP [i211]). The measurements were performed only on patients who had negative measurements for rVes v 5 CAP.
 - Forty-seven patients were also measured IgE against rApi m 1 allergen and additional 50 with IgE against rApi m 2 allergen (LITE).

We also tested 49 healthy controls, both with rApi m 1 and with rVes v 5 on LITE and CAP systems.

In all of the above measurements, values > 0.35 kU/L were considered positive.

Additionally, the measurements of total IgE values were conducted on all patients using CAP.

Calculations of the sensitivities of different diagnostic tests

As all patients were sensitized to bee or yellow jacket venom and all had suffered an allergic reaction after a corresponding bee or yellow jacket sting, all patients were considered allergic to honey bee or yellow jacket venom. Thus, the sensitivities of different methods were calculated as follows:

- In the sample of patients allergic to yellow jackets: the number of patients positive with a particular test in a yellow jacket-allergic sample/number of all patients in a yellow jacket-allergic sample.
- In the sample allergic to bees: the number of patients positive with a particular test in a bee-allergic sample/number of all patients in a bee-allergic sample.

The sensitivities of rApi m 1 and rVes v 5 with both techniques were also calculated for each of the reaction severity group subsamples.

Correlations between different diagnostic methods

Correlation coefficients between the results of the CAP and LITE techniques on both samples [rApi m 1 CAP vs. rApi m 1 LITE (bee-allergic sample) and rVes v 5

CAP vs. rVes v 5 LITE (yellow jacket-allergic sample)] were calculated. Because the data were not normally distributed (Saphiro–Wilk test), Spearman's correlation coefficient was used.

The status of the result (positivity/negativity) using the LITE technique was calculated on a subsample of patients who were CAP negative (rApi m 1 LITE status on a subsample of rApi m 1 CAP-negative patients; rVes v 5 LITE status on a subsample of rVes v 5 CAP-negative patients) or vice versa.

The linear regression between CAP and LITE results was performed on a subsample of patients who had positive results for measurements with both techniques, and the values of measurements with both techniques were below 100 kU/L.

Prediction of reaction severity

The values of different diagnostic methods of the IgE system (values of total IgE results, values of sIgE results, values of results of IgE against rVes v 5 and rApi m 1 using LITE), the demographics of patients (age, gender) and the source insect were used as predictors of the severity of the reaction with multiple logistic regression to establish the independent correlation of each factor to the severity of the reaction. Two logistic regression models were constructed. In the first model, the non-local reaction (SR or AR) was considered to be the positive outcome of the dependent variable (the severity) and the LR was considered to be the non-positive outcome of the dependent variable. This model was fitted on the combined sample of yellow jacket- and bee-allergic patients. In the second model, the AR was considered to be the positive outcome and the SR to be the non-positive outcome of the dependent variable (the severity). The second model was fitted on a combined sample of yellow jacket- or bee- allergic patients who suffered a SR or an AR (patients that suffered a LR were not used as observations to fit the second model).

Data analysis

All data analysis including multiple logistic regression was performed using R [14]. Sample proportions were compared with each other using the chi-squared test (with Yates's continuity correction where appropriate). The 95% CI for sample proportions was calculated using the exact binominal test. The correlations were calculated using Spearman's correlation coefficient. To check the normality of the distribution of a specific parameter, the Shapiro–Wilk test was used. P-values of < 0.05 were considered statistically significant.

Results

Calculations of sensitivities of different diagnostic tests

The results of sensitivities of different diagnostic tests (and their combinations) are presented in Fig. 1. The sensitivities of species-specific major antigens (SSMA; rVes v 5 and rApi m 1) using the LITE system were significantly higher in both samples when compared to the CAP system [88% (LITE) vs. 71% (CAP), $P = 0.004$ on a bee venom-allergic sample, and 93% (LITE) vs. 82% (CAP), $P = 0.026$ on a yellow jacket venom-allergic sample]. The addition of another recombinant allergen (rApi m 2 and rVes v 1) increased the sensitivity even further [95% in the bee venom-allergic sample (using LITE) and 90% in the yellow jacket venom-allergic sample (using CAP)], but this increase in sensitivity was not statistically significant. In the yellow jacket venom-allergic sample, the combined sensitivity of both

recombinant allergens (rVes v 5 and rVes v 1) using the CAP system was lower than the sensitivity of just rVes v 5 using the LITE system. In addition, the combined sensitivity of rApi m 1 CAP and rApi m 2 LITE was lower than just that of rApi m 1 LITE (86% vs. 88%). However, these differences were not statistically significant. It is also interesting to note that the combined sensitivity of rVes v 5 LITE and rVes v 1 CAP almost reached the sensitivity of native yellow jacket venom (97%; 95% CI = 92–99%). The combination of recombinant allergens using different systems [rApi m 1 (CAP) and rApi m 2 (LITE)/rVes v 5 (LITE) and rVes v 1 (CAP)] were calculated, because the rApi m 2 allergen is not available for CAP technique, and rVes v 1 allergen is not available for LITE technique.

The results of the diagnostic sensitivities of rApi m 1 and rVes v 5 with both techniques on subsamples of patients with different reaction severities are presented in Table 1. It can be seen that the differ-

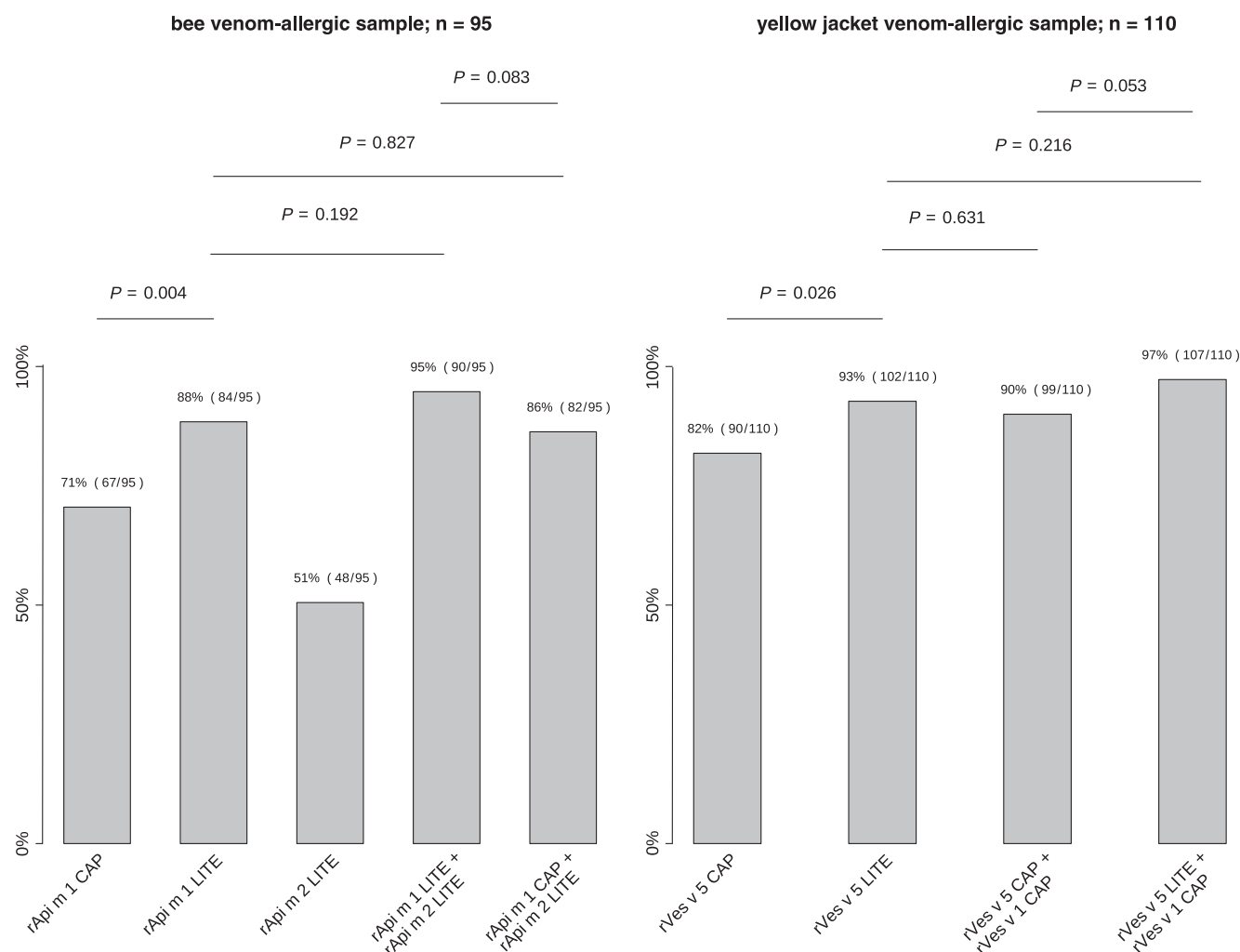


Fig. 1. Sensitivities of different diagnostic methods on bee venom-allergic sample ($n = 95$) and on yellow jacket venom-allergic sample ($n = 110$). The sensitivities of rApi m 1 and rVes v 5 differed significantly between CAP and LITE technique.

Table 1. Sensitivity of both techniques with rVes v 5 and rApi m 1 in subsamples of patients with different reaction severities (LR = local reaction, SR = systemic reaction, AR = anaphylactic reaction)

Reaction severity	Sensitivity (CAP) (%)	Sensitivity (LITE) (%)	Difference in sensitivity, %	P-value
Bee venom-allergic patients (n = 95)				
LR	9/13 (69)	10/13 (76)	6	1
SR	18/34 (53)	26/34 (76)	23	0.08
AR	40/48 (83)	48/48 (100)	17	0.01
Wasp venom-allergic patients (n = 110)				
LR	10/12 (83)	11/12 (92)	9	1
SR	31/36 (86)	35/36 (97)	11	0.2
AR	49/62 (79)	56/62 (90)	11	0.13

ence in sensitivities was higher in patients with more severe reactions (AR + SR). The significance level of the sensitivity difference was most pronounced in a subsample of bee-allergic patients with an AR, in which rApi m1 CAP measurements recognized 40 of 48 patients (83%) and rApi m 1 LITE measurements recognized 48 of 48 (100%; $P = 0.01$) patients in a subsample.

Negative rApi m 1 and rApi m 2 levels were measured in 46 of 47 and 48 of 50 of wasp venom-allergic individuals, thus achieving the specificity of 98% and 96%.

Three of 49 healthy controls were positive for rApi m 1 measurements on LITE system and 1 of 49 on CAP system yielding the specificities of 94% and 98%. The results were similar for rVes v 5 measurements where 2 of 49 healthy controls were positive on LITE and again 1 on CAP system (specificities of 96% and 98%, respectively).

Correlations between different diagnostic methods

The scatterplots of log values measured with CAP and LITE with a fitted linear regression line of logged results [on a subsample of patients who had positive results using both techniques (CAP and LITE), and whose results using both techniques were < 100 kU/L] are presented in Fig. 2 (on a bee-allergic subsample) and Fig. 3 [on a yellow jacket-allergic subsample (please note that log results are presented only in the Figs 1 and 2 for better visual representation of the results that

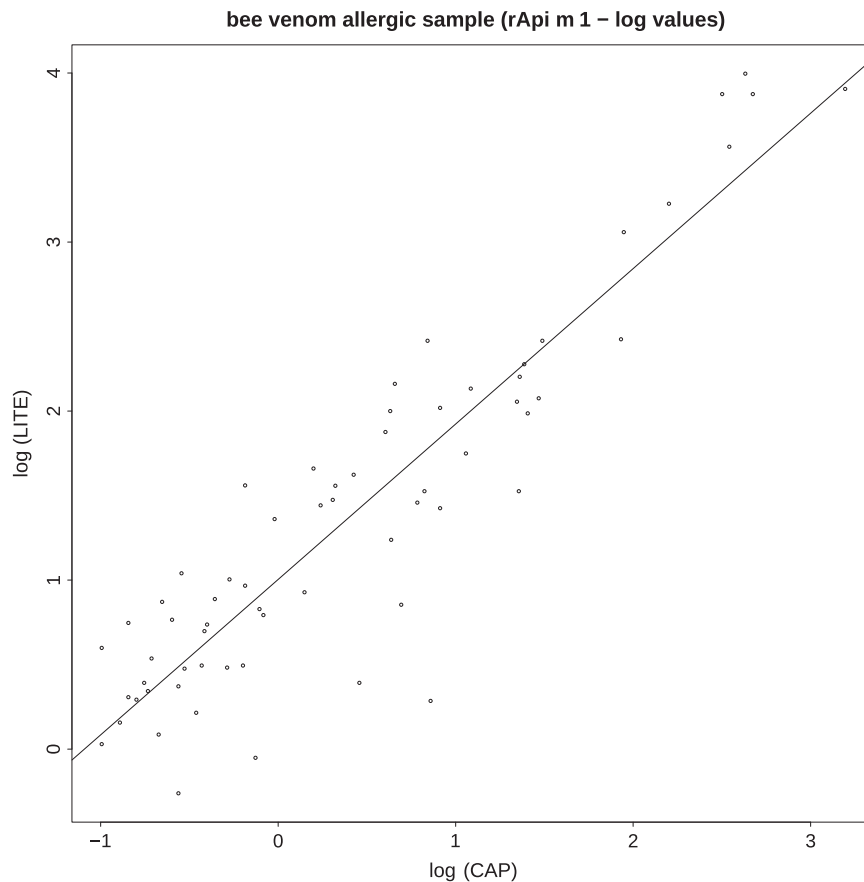


Fig. 2. Scatterplot of log rApi m 1 values measured with CAP and LITE technique with a fitted regression line (of logged results) on a subsample of bee venom-allergic patients whose values of rApi m 1 CAP and rApi m 1 LITE were > 0.35 kU/L and < 100 kU/L ($R^2 = 0.88$).

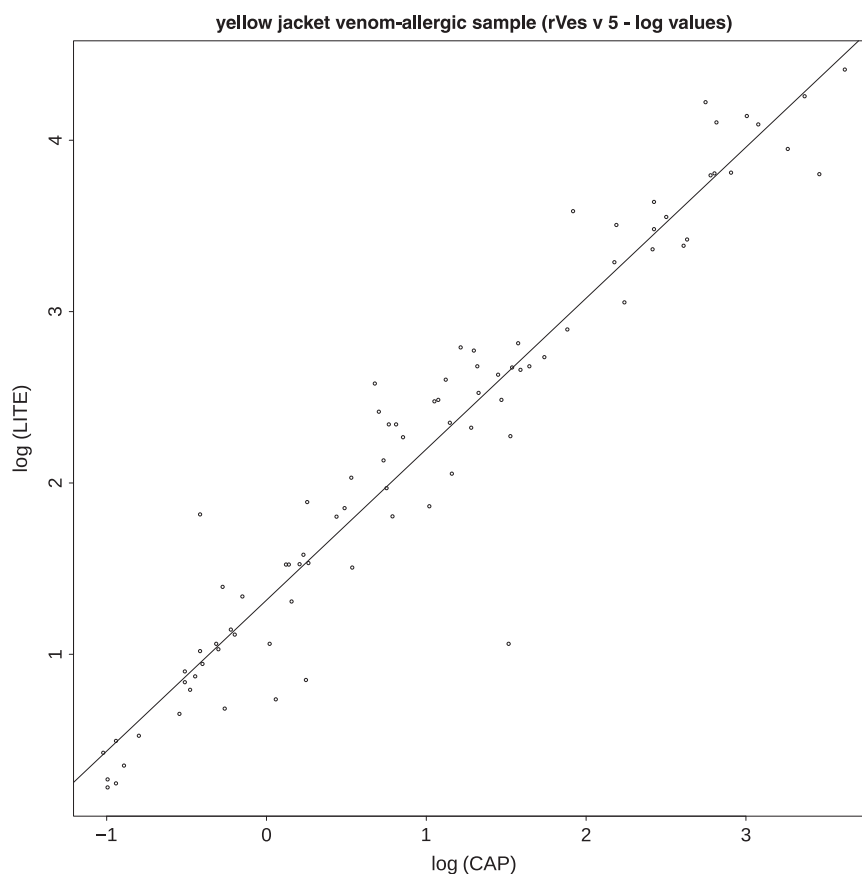


Fig. 3. Scatterplot of log rVes v 5 values measured with CAP and LITE technique with a fitted regression line (of logged results) on a sample of yellow jacket venom-allergic patients whose values of rVes v 5 CAP and rVes v 5 LITE were > 0.35 kU/L and < 100 kU/L ($R^2 = 0.87$).

are clustered at lower values; when discussing linear regression coefficients, they are referring to the non-logged results)].

The Spearman's correlation coefficient rho (as the data were not normally distributed) between CAP and LITE measurements on both samples was higher than 0.9 [0.93 in the bee-allergic sample ($P < 0.001$) and 0.98 in the yellow jacket-allergic sample ($P < 0.001$)], suggesting a very strong positive correlation. Also, the results of R^2 for linear regression on two subsamples (described above) indicate a very strong dependency between both variables, as more than 85% of one variable can be explained by the variability of the other on both subsamples. The coefficients of linear regression were 2.7 for rApi m 1 and 2.3 for rVes v 5. The numbers of patients with positive/negative results for IgE measurements against recombinant SSMA (Api m 1 and Ves v 5) with the CAP/LITE technique are presented in Table 2. Interestingly, approximately 60% of patients who had negative results for recombinant SSMA measurements with the CAP technique had simultaneously positive results for recombinant SSMA measurements with LITE; by contrast, none of the patients who had

Table 2. Number of patients positive/negative for rApi m 1/rVes v 5 allergen with each of the techniques

	LITE positive	LITE negative
Bee venom-allergic patients ($n = 95$; [rApi m 1])		
CAP positive	67	0
CAP negative	17	11
Wasp venom-allergic patients ($n = 110$; [rVes v 5])		
CAP positive	90	0
CAP negative	12	8

negative results for recombinant SSMA measurements with LITE had positive results for recombinant SSMA measurements with CAP. This was apparent in both samples.

As both assays measure down to values of 0.1 kU/L, the agreement between the two assays were separately measured on patients whose CAP measurements were between 0.1 kU/L and 0.35 kU/L (despite the fact that both manufacturers state that the cut-off for positivity is > 0.35 kU/L). The correlation coefficients rho were again high (rho = 0.91 in a bee-allergic subsample; rho for = 0.94 in a yellow jacket-allergic subsample). The

linear regressions coefficients (LITE measurements as dependent variable; CAP measurements as independent variable) were 4.9 on a bee-allergic subsample and 3.6 on a yellow jacket-allergic subsample. All of the coefficients (rho and linear regression coefficients) were statistically significant.

Prediction of reaction severity

The results of the logistic regression models are shown in Table 3. In both models [using the SR + AR as the positive and LR as the non-positive outcome of the dependent variable (model 1) and using the AR as the positive and SR as the non-positive outcome of the dependent variable (model 2)] showed that of all the predictors that were used to predict the severity (age, gender, total IgE, sIgE, recombinant antibodies and the source insect) of the reaction, the only independent statistically significant predictor of the severity of the reaction was age. In model 1, the OR for the age predictor was 1.04 ($P = 0.011$), and in model 2, the OR for age was 1.02 ($P = 0.039$).

Discussion

In the current study, we showed that the measurements of IgE antibodies against recombinant venom allergens with Immulite have a significantly higher sensitivity for diagnosing Hymenoptera venom allergy than the measurements of the same antibodies with ImmunoCAP, while the specificity of both assays is comparable. Moreover, the combination of rApi m 1 and rApi m 2 with LITE (95%) and rVes v 5 with LITE and rVes v 1 with CAP (97%) almost reached the sensitivities of native venoms. In addition, we were not able to show

that levels of IgE antibodies against venom recombinants correlate with the severity of the reaction, the same that was observed for the levels of IgE antibodies against native venoms [15].

The use of rApi m 1 and rVes v 5 was a major breakthrough in the diagnosis of Hymenoptera venom hypersensitivity because the preparations are devoid of cross-reactive carbohydrate epitopes, and therefore, the double positivity observed due to the response of IgE antibodies against those epitopes in native Hymenoptera venom preparations was resolved, improving the diagnostic process. However, the question of insufficient sensitivity of the preparations arose because the sensitization pattern with respect to various venom components differs between Hymenoptera-allergic patients. The problem of inadequate sensitivity of SSMAAs is particularly concerning in honeybee venom allergy, because it was shown [6] that as few as 57% of patients who had an allergic reaction after a bee sting and positive sIgE measurements against native bee venom also had positive IgE antibodies against rApi m 1. Because of the inadequate sensitivity of recombinant SSMAAs, other recombinant allergens (rApi m 2 and rVes v 1) were added to the standard battery of recombinant allergens for use in routine clinical practice.

To our knowledge, only one study [8] has evaluated the use of multiple recombinant allergens in Hymenoptera venom-allergic patients. Unfortunately, in this study [8], authors used custom CAP venom recombinants which are not available for routine diagnostic use and thus are impossible to be tested by other groups.

We therefore sought to evaluate the utility of currently available recombinant allergens in Hymenoptera venom allergy with both routinely used systems (ImmunoCAP and Immulite 2000), as a recent study with venom extracts on those two systems showed that their sensitivities might differ [16].

The sensitivities of IgE antibodies against SSMAAs with CAP and LITE were calculated first. On a sample of bee venom-allergic patients, the sensitivity of rApi m 1 with CAP was 71%. This supported the concerns raised by other studies [5–7] that the detection of sIgE against rApi m 1 with CAP is a valuable but not sufficient diagnostic tool in diagnosing Hymenoptera venom allergy. The detection of rApi m 1 with LITE on the same sample significantly improved (88%, $P = 0.004$) the sensitivity, and the combined use of both routinely used/manufactured recombinant proteins (rApi m 1 and rApi m 2), with LITE, recognized almost all the patients (95%; 95% CI = 88–98%) in the sample, thus equalling the sensitivity of an extended (six different) array of Api m allergens (94%) [8]. Very important, the LITE rApi m 1 allowed unambiguous identification of all (100%) honeybee-allergic patients in an anaphylactic reaction subsample in comparison with sensitivities of

Table 3. Results of coefficients of logistic regression models. Only age independently statistically significantly correlates with reaction severity in both models

Predictor	OR	95% CI for OR		P-value
Logistic regression (local reaction [0] vs. non-local reaction {systemic or anaphylactic} [1])				
Rec. venom IgE	1.022	0.993	1.058	0.17
Age	1.043	1.011	1.080	0.01*
Native venom IgE	0.974	0.940	1.010	0.13
Total IgE	0.999	0.996	1.004	0.80
Sex	1.500	0.619	3.709	0.37
Source insect (W)	1.234	0.498	3.112	0.65
Logistic regression (systemic reaction [0] vs. anaphylactic reaction [1])				
Rec. venom IgE	0.982	0.962	1.001	0.08
Age	1.024	1.003	1.048	0.04*
Native venom IgE	1.012	0.984	1.042	0.42
Total IgE	1.002	0.998	1.006	0.35
Sex	1.392	0.743	2.624	0.30
Source insect (W)	1.643	0.839	3.261	0.15

83% with the CAP rApi m 1 allergen ($P = 0.01$). These high sensitivities are comparable with previous *in vivo* skin tests [9] or *in vitro* [2, 10, 11] evaluation of rApi m 1 IgE reactivity either with custom IgE immunoblotting and/or ELISA [10, 11] or with the discontinued ADVIA system (Siemens AG). Interestingly, the inconsistency in diagnostic sensitivity started with the reporting of rApi m 1 ImmunoCAP IgE testing [5–8] and currently, there is no clear explanation for such a discrepancy. Importantly, the specificities of both assays (LITE and CAP) for rApi m 1 as well as for rVes v 5 were high (from 94 to 98%), which suggests that better sensitivity of the LITE system was not due non-specific bounding of IgE antibodies.

The sensitivity of rVes v 5 on a sample of patients allergic to yellow jacket venom using the CAP technique was 82%. Again, as for LITE rApi m 1, significantly improved sensitivity (93%; $P = 0.026$) with LITE was also shown for rVes v 5 testing. Surprisingly, the combined sensitivity of two routinely produced reagents (rVes v 5 and rVes v 1) for ImmunoCAP was measured and calculated, and it did not reach the sensitivity of only rVes v 5 with LITE (90% vs. 93% $P = 0.63$). However, this difference was not statistically significant. This improved utility of both honeybee and yellow jacket venom recombinants implicated that if whole venom-specific IgE measurement or skin testing, which include all the principal allergens, demonstrated inconclusive results or the sting history is equivocal, the testing with the individual components should be performed as a part of diagnostic algorithm.

The correlation of the results of measurements of both recombinant SSMA (rApi m 1 and rVes v 5) using LITE and CAP was then calculated. As the data were not normally distributed, Spearman's correlation coefficient was used. Both correlation coefficients were, as expected, statistically significantly ($P < 0.001$ for both) very high and positive ($\rho = 0.98$ for rVes v 5 preparations and 0.93 for rApi m 1 preparations). We also fitted a linear regression line and calculated R^2 for measurements of the same SSMA with CAP and LITE, using one as the predictor and the other as the outcome variable (CAP measurements as the predictor and LITE measurements as the outcome variable) on a subsample of patients who had positive values for the tests using both techniques. The R^2 of both subsamples was around 0.85, thus concluding that 15% of variability of measurements with LITE technique cannot be explained by the variability of measurements with CAP technique. It is also important to note the coefficient of linear regression lines. The coefficient for rApi m 1 is 2.7 and for rVes v 5 is 2.3, indicating that the results of rApi m 1 LITE in an average patient are 2.7-times higher than the results of that patient's rApi m 1 CAP measurements. This result also means that the results of

rVes v 5 LITE in an average patient are 2.3-times higher than the results of that patient's rVes v 5 CAP measurements. These observations and the increased sensitivity of LITE may be due to differences in the detection (calibration/interpolation algorithm), differences in the measurement technique used or differences in allergen preparations used by both systems to bind available IgE antibodies.

For ImmunoCAP preparations, the manufacturer states that they are a cross-reactive carbohydrate determinant (CCD)-free component, but it does not reveal in which expression system the preparations are produced. For Immulite preparations, the manufacturer states that they are cloned and expressed using Sf9 insect cells infected by a recombinant baculovirus.

Because the production system of the ImmunoCAP preparations is not known, one can speculate that the difference between the sensitivities and also the higher measurements in the LITE system might be related to differences in the activity of recombinant venom allergens [17], which can be the result of their production either in bacterial expression systems as non-glycosylated allergens or in different eukaryotic systems as glycosylated allergens [17, 18]. Nevertheless, we are more prone to the idea that the CAP system is lacking the binding capability of available IgE antibodies or that there are changes in the interpolation calibration algorithm between the two assays, as the measurement results were about 2.5 times higher (roughly equal) for both recombinant allergens (rApi m 1 and rVes v 5) on LITE compared to the CAP system. The on average higher measurement values of the LITE system were even more apparent at lower concentrations of IgE antibodies. In patients whose CAP measurements were between 0.1 and 0.35 kU/L, on average, the values of rApi m 1 measured with LITE were 4.9 times higher than the values of rApi m 1 measured with CAP, and also, the values of rVes v 5 measured with LITE were 3.6 times higher than the values of rVes v 5 measured with CAP.

As just three controls had values of rApi m 1 CAP higher than 0.1 and just another 3 had values of rVes v 5 CAP higher than 0.1 (just 4 rApi m 1 LITE and 6 rVes v 5 LITE), we were not able to draw the receiver operator characteristic (ROC) curves to choose the best cut-off value and also to compare the values of areas under the curve (AUCs) for those ROC curves.

Because the LITE system measures on average higher values, the trend of which was also apparent in 10 controls [in all 10 controls with values of LITE higher than 0.1 kU/L (all 6 that had CAP values higher than 0.1 are included), the LITE measurements were higher than the CAP measurements ($P = 0.002$ for that being just coincidence)], the calibration interpolation differences between the two assays can be the source of the dis-

crepancy. But nevertheless, as the cut-off is set as it is, by the manufacturers of the assays, the specificities of both assays are comparable, but the sensitivities of the CAP are significantly lower.

Finally, we attempted to predict whether some components of the IgE system (total IgE levels, levels of IgE antibodies against native venoms and levels of IgE antibodies against recombinant SSMAAs using the LITE technique), especially the recombinants, together with some of the demographic (age, sex) data and data regarding the reaction (source), could independently predict the severity of the reaction, as some publications [13] found that higher total IgE values are associated with milder reaction severities. Thus, we fitted two logistic regression models with the above variables as predictors. The binary outcome in the first model was non-local reactions (SR + AR) and local reaction (LR), and the binary outcome in the second model was anaphylactic reaction (AR) and systemic reaction (SR). In both models, only age was the independent statistically significant predictor of reaction severity (OR in model 1 = 1.04, $P = 0.011$; OR in model 2 = 1.02, $P = 0.039$). This result was expected because old age is usually associated with a higher probability of cardiovascular and/or pulmonary diseases and

because the presence of disease in those organ systems can result in more rapid decompensation of the organ systems after an insult (allergic reaction). Therefore, symptoms of a more severe reaction and drugs used to treat cardiovascular diseases (B-blockers) can oppose the action of anti-anaphylactic hormones (adrenaline).

To show the true independency [19] of the statistically significant correlation of age with reaction severity, we should have adjusted the above-mentioned variables (presence of cardiopulmonary disease and use of cardiovascular medicine) in the models, but we did not collect those two variables in patients. Nevertheless, none of the models showed any statistically significant correlation between either part of the IgE system and reaction severity.

Collectively, improved sensitivities of rApi m 1 and rVes v 5 recombinant venom allergens on LITE system should facilitate more accurate serologic dissection of bee and yellow jacket allergy.

Conflicts of interest

The authors declare no conflict of interest.

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