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CLINICAL STUDY

Basophil activation test in the diagnostics of hymenoptera venom allergy

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ABSTRACT

OBJECTIVES: The aim of the study was to analyse the diagnostic performance of the basophil activation test (BAT), to compare the diagnostic reliability of BATs performed with different types of allergens, which are available in Slovakia and to verify the correlation between the symptom severity and the sensitivity and specificity of the BAT in 114 patients with suspected hymenoptera venom allergy (HVA).

BACKGROUND: Diagnosis of the HVA and the identification of the appropriate venom for immunotherapy are in Slovakia based on detailed patient'medical history, skin tests and detection of sIgE. In unclear cases, where the clinical decision regarding the relevant insect species for immunotherapy is difficult, the cellular tests are recommended in several countries, such as Sweden, Spain, Germany, Denmark and Italy. In Slovakia, BAT is not adapted as s part of routine diagnostic work-up.

METHODS: The identification of the culprit hymenoptera species among 114 patients was based on detailed history, skin tests and detection of sIgE. Obtained results were compared with the results acquired by the BAT.

RESULTS: The sensitivity of the BAT was 80.8 % and the specificity was 87.8 %. The sensitivity of the BAT was higher when using Soluprick SQ Allergens, but the specificity was higher with BŰHLMANN CAST Allergens. In the study no correlation between the symptom severity and the sensitivity and specificity of the BAT was observed.

CONCLUSIONS: The results show that the BAT can be recommended in the identification of the appropriate venom for immunotherapy, the only specific treatment that is currently available for patients with HVA. Allergen source is one of critical factors in diagnostic reliability of the BAT *(Tab. 4, Ref. 29)*. Text in PDF *www.elis.sk*

KEY WORDS: hymenoptera venom allergy, allergy diagnosis, basophil activation test, sensitivity, specificity.

Introduction

Hymenoptera venom allergy (HVA) is the most common cause of anaphylactic reactions in adults and the second-most common cause in children, after food-related anaphylaxis (1, 2). In European epidemiological studies the sensitization rate to honeybee or wasp venom in the general population varies between 27.1–44.1 % (3). Strong local reactions occur in 2.4 to 26.4 % of general population. The prevalence of systemic reactions ranges between 0.3 and 7.5 % in adult population (4). The estimated number of annual mortalities due to insect sting-induced anaphylaxis ranges from 0.03 to 0.45 per one million inhabitants (5) Due to the fear of future reactions, HVA imposes a significant impact in health-related Quality of Life (6, 7).

The management of HVA includes short-term interventions to treat acute reactions and long-term strategies to minimize the risk of consecutive reactions. Acute reactions are managed by symptomatic treatment. Long-term care includes patient education on how to minimize exposure to further stings, prescription of an emergency kit for self-treatment and venom immunotherapy (VIT) (8). VIT is the only specific treatment that is currently available for patients with a history of systemic reaction to a hymenoptera insect sting (9). VIT with the responsible hymenoptera venoms is highly effective in protecting patients with hymenoptera venom allergy including anaphylaxis. It has proven to be effective in 95 % of patients allergic to wasp venom and between 75 and 85 % of those allergic to honeybee venom (10). Identification of the appropriate venom for VIT is often hampered by a high frequency of double sensitizations to honeybee and wasp venom. Depending on the method used, up to 64 % of insect venom-allergic patients show double-positivity. Double sensitization could be caused by true double sensitizations, cross-reactive specific IgE (sIgE) antibodies that recognize similar protein epitopes of hyaluronidase, vitellogenin or dipeptidylpeptidase of honeybee and wasp venom, or the presence of sIgE to cross-reactive carbohydrate determinations (CCD) in both venom allergens (11). It is pondered that IgE directed to CCD is the major cause of multiple sensitization detection (12). Real double sensitization to both venoms indicates

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potential systemic allergic reactions to the next sting by either insect species, if not treated by immunotherapy with both venoms. In the case of cross-reactions as a cause for double positivity, the treatment with the venom of the primarily responsible insect alone would be sufficient and more cost effective (13).

In Western and Central Europe sting reactions are primarily due to sting by certain social wasps (*Vespula vulgaris* and *Vespula germanica* in particular) and to honeybees (*Apis mellifera*). Paper wasp species are very common in Southern Europe, the US and in tropical and subtropical areas (especially South America) (1, 14). The identification of the appropriate venom for VIT is based on detailed history, venom skin tests and detection of hymenoptera sIgE. The determination of sIgE by component-resolved diagnostic test (CRD) allows a more precise characterization of the sensitization profile of an individual patient. Cellular tests, like the basophil activation test (BAT), are recommended in unclear cases where the decision regarding the relevant insect species for immunotherapy is difficult.

The BAT reports both high sensitivity (85–100 %) and specificity (83–100 %) to diagnose hymenoptera venom allergy (15, 16). Its high specificity is based on monitoring cellular response following antigen stimulation in real time (10). Basophils with their high-affinity IgE receptors are easily accessible and, therefore, they can be used as indicator cells for IgE-mediated reactions. Upon allergen challenge and cross-linking of membrane-bound IgE antibodies, basophils up-regulate certain activation markers on their surface, such as CD63 and CD203c, as well as intracellular molecules, such as p38 mitogen activated protein kinase (17). The BAT uses flow cytometry to measure the expression of these activation markers.

The aim of the following prospective study was to analyse the diagnostic reliability of the BAT among selected patients with suspected hymenoptera venom allergy. Furthermore, the study compares the diagnostic performance of BAT performed with different allergens, which are available in Slovakia: commercial venom extracts for prick testing Soluprick SQ, ALK-Abelló, Denmark and BŰHLMANN CAST Allergens, Germany, optimized for *in vitro* use in the cellular allergy assays. In addition, the study compares the sensitivity and specificity of BAT performed with honeybee venom and BAT performed with wasp venom, and assess the correlation between the severity of symptoms and the sensitivity and specificity of the BAT.

Patients and methods

A total of 114 consecutive patients (47 men, 67 women) between the ages of 6 and 72 years (average age 47.5 ± 14.66 years) with suspicion of HVA were evaluated in the Outpatient Department of Dermato-allergology, Department of Dermatovenerology, University Hospital, Bratislava, Slovakia between 2014 and 2019. Laboratory diagnostic trsts were performed in the Department of Clinical Immunology and Allergology, Oncology Institute St. Elizabeth, Bratislava, Slovakia.

Of the 114 evaluated patients 81 had a documented history of systemic allergic reaction (SR) after hymenoptera sting as follows: 38 experienced a grade I reaction, 23 experienced a grade II reaction, 14 experienced a grade III reaction, and 6 of them experienced a grade IV reaction, according to H. L. Mueller (18). 24 patients experienced strong local reactions. Nine patients had no reaction after the sting. All of them were participants in a beekeeping course, which required the results of allergic testing against hymenoptera allergens.

The time interval from the last sting to examination was between 1 and 52 months (average time interval 10.2 ± 3 months) for 85 patients. The exact time interval was not given in 20 patients (time interval 10 or more years). Nine patients, participants in a beekeeping course, had no history of stings in the past.

All individuals enrolled in the study were informed and signed the informed consent. The approval number from the Ethical committee of the Old Town Hospital and the Faculty of Medicine of Comenius University, Bratislava, Slovak Republic is 25/2021.

The identification of the culprit hymenoptera species was based on detailed history, venom skin tests and detection of hymenoptera venom-specific IgE. A combination of these tests improved the diagnostic precision in HVA (10). The results of these tests were compared with the results of the BAT. Skin testing and blood sampling for *in vitro* testing were realized in one session.

In particular, patients with systemic mastocytosis or patients with high serum tryptase level (> 11.4 ng/mL) are at an increased risk of frequent and severe episodes of anaphylaxis, therefore in all involved patients the basal serum tryptase level was determined (19).

Patient´s history

All patients were questioned for severity of symptoms and, in particular, for the type of insect responsible for the sting. The verification of a previous reaction after sting by clinical history should build the basis for subsequent diagnostic work-up since asymptomatic sensitization to Hymenoptera venom is observed frequently (5).

Skin tests

In all patients, skin sensitivity to both venoms (honeybee and wasp) was determined by a skin prick test (SPT). In several countries skin testing is considered the gold standard, because they are easy to conduct, fast and relatively inexpensive and can provide helpful diagnostic information (20, 21). SPTs were performed with commercial venom extracts for prick testing Soluprick SQ, ALK-Abelló, Denmark. The venoms, honeybee venom (*Apis mellifera*) and wasp venom (Vespula species) were tested. Histamine dihydrochloride 10 mg/mL and physiological saline were used as positive and negative controls, respectively. Testing was done with incremental concentrations of 10 µg/mL, 100 µg/mL and 300 µg/mL. Positive results were defined as a wheal ≥3 mm diameter with a flare 15–20 minutes after the SPT. No systemic reactions have occurred following SPTs.

Determination of specific immunoglobin E antibodies (sIgE)

Detecting sIgE in conjunction with the patients´s clinical history is one of the main diagnostic methods in HVA (20). In all patients,

sIgE to whole insect venoms of honeybee, Vespula spp. (Yellow jacket), *Vespa crabro* (Hornet), Polistes spp. (European wasp), *Dolichovespula arenaria* and *Dolichovespula maculata* (Bald-faced hornet) were determined by standardized solid phase enzyme immunoassay ELISA (Enzyme-linked immunosorbent assay), Hytec 288, Hycor, USA. The recombinant venom allergens used were rApi m 1 (phospholipase A2 – Apis mellifera), rVes v 1 (phospholipase A1 – Vespula vulgaris), rVes v 5 (antigen 5 – Vespula vulgaris) and the MUXF3 carbohydrate epitope from bromelain. Bromelain extracted from pineapples is a glycoprotein commonly used for reference to sIgE-CCD detection and analysis. The diagnosis was established by the use of FEIA (Fluorescent enzyme immunoassay), ImunoCAP Phadia250 (Thermo Fisher), Sweden. A value of 0.35 ≥ kU/L was considered positive.

Basophil activation test

The BAT was performed with the kit BasoFlowEx Kit, Exbio, Czech Republic. In the first 84 cases, the allergens used were commercial venom extracts (Soluprick SQ, ALK-Abelló, Denmark) with bee venom (*Apis mellifera*) and wasp venom (Vespula species), all in 3 different concentrations (10 µg/mL, 100 µg/mL and 300 µg/mL). In the next 30 cases, the allergens used were BŰHLMANN CAST Allergens, Germany: BAG2-I1 Honey Bee Venom and BAG2-I3 Yellow Jacket Venom, both in a final concentration of 11.5 ng/mL. The analysis was performed on BD FACSCanto II flow cytometer, Becton Dickinson Bioscience, San Jose, California, USA.

The analyses were performed within 8 hours of blood sampling to heparinized tubes. One hundred µL of the whole blood was incubated with 100 µL of stimulation buffer (BasoFlowEx Kit) containing IL-3 to enhance the allergen-specific up-regulation of CD63, and 10 µL of allergen for 15 minutes at 37 °C. The positive control was prepared with stimulation buffer and 10 µL of the stimulation control (BasoFlowEx Kit) containing chemotactic peptide fMLP (N-formyl-methionyl-leucyl-phenylalanine). Negative control contained only the stimulation buffer. Subsequently 20 µL of Staining Reagent (BasoFlowEx Kit) containing monoclonal antibodies was added to each sample. The antibodies included were anti-CD63 labelled with fluorochrome FITC (Fluorescein isothiocyanate) for measuring basophil activation and anti-CD203c labelled with fluorochrome PE (Phycoerythrin) for gating the basophil population as well as measuring basophil activation. After staining, the samples were incubated for 20 minutes at 4 °C, lysed for 10 minutes (Lysing Solution, BasoFlowEx Kit), centrifuged at 300xg for 5 minutes and washed. After decanting the supernatant, the samples were re-suspended in 300 µL of PBS (Phosphate-buffered saline) and analysed within 2 hours using flow cytometry.

Tab. 1. Diagnostic performance analysis of BAT.

	BAT bee	BAT wasp	BAT total
Prevalence[a] (%)[b]	13.6 (7.6–20.8)	55.3 (45.7–64.6)	64.0 (54.5–72.8)
PPV (%)	52.4 (27.8–74.3)	83.9 (72.3–91.9)	92.2 (82.7–97.4)
NPV (%)	95.7 (89.4–98.2)	78.8 (65.3–88.9)	72.0 (57.5–83.8)
Sensitivity (%)	73.3 (44.9–92.2)	82.5 (70.9–90.9)	80.8 (69.9–89.1)
Specificity (%)	89.9 (82.2–95.1)	80.4 (66.9–90.2)	87.8 (73.8–95.9)
LR+	7.26 (3.68–13.86)	4.21 (2.50–7.56)	6.63 (3.13–15.3)
LR–	0.29 (0.12–0.58)	0.22 (0.12–0.36)	0.22 (0.13–0.34)
DOR	24.47	19.38	30.34

[a] Prevalence in the target population of patients with clinical suspicion of HVA.
[b] All proportions are expressed as percentages together with 95 % confidence intervals
BAT bee – BAT performed with honeybee venom (irrespective of the type of allergen: Soluprick or BŰHLMANN), BAT wasp – BAT performed with wasp venom (irrespective of the type of allergen: Soluprick or BŰHLMANN), BAT total – results of BAT irrespective of the type of allergen (bee venom or wasp venom, Soluprick or BŰHLMANN), PPV – Predictive value of +ve test (post-test likelihood of disease), NPV – Predictive values of -ve test (post-test likelihood of no disease), LR+ – Likelihood Ratio positive test, LR- – Likelihood Ratio negative test, DOR – Diagnostic Odds Ratio.

Tab. 2. Diagnostic performance analysis of BAT performed with Soluprick SQ allergens.

Soluprick SQ	BAT bee	BAT wasp	BAT total
Prevalence[a] (%)[b]	14.3 (7.6–23.6)	52.4 (41.2–63.4)	64.7 (49.5–71.2)
PPV (%)	52.6 (28.8–75.5)	80.0 (66.3–89.9)	90.2 (78.6–96.7)
NPV (%)	96.9 (89.3–99.6)	88.2 (72.5–96.7)	84.8 (68.1–94.9)
Sensitivity (%)	83.3 (51.6–97.9)	90.9 (78.3–97.5)	90.2 (78.6–96.7)
Specificity (%)	87.5 (77.6–94.1)	75.0 (58.8–87.3)	84.9 (68.1–94.9)
LR+	6.67 (3.39–12.83)	3.64 (2.23–6.45)	5.95 (2.89–15.3)
LR–	0.19 (0.05–0.51)	0.12 (0.05–0.29)	0.11 (0.05–0.25)
DOR	35.0	30.0	51.5

[a] Prevalence in the target population of patients with clinical suspicion of HVA.
[b] All proportions are expressed as percentages together with 95 % confidence interval
BAT bee – BAT performed with honeybee venom, BAT wasp – BAT performed with wasp venom, BAT total – BAT performed with Soluprick SQ allergens (irrespective of the type of allergen: bee venom or wasp venom), PPV – Predictive value of +ve test (post-test likelihood of disease), NPV – Predictive values of -ve test (post-test likelihood of no disease), LR+ – Likelihood Ratio positive test, LR- – Likelihood Ratio negative test, DOR – Diagnostic Odds Ratio.

Tab. 3. Diagnostic performance analysis of BAT performed with BŰHLMANN CAST Allergens.

BŰHLMANN CAST	BAT bee	BAT wasp	BAT total
Prevalence[a] (%)[b]	10.0 (2.1–26.5)	63.1 (43.8–79.9)	73.1 (53.9–87.5)
PPV (%)	50.0 (1.3–98.7)	99.2 (72.4–100.0)	99.2 (74.2–100.0)
NPV (%)	92.9 (76.5–99.1)	61.1 (35.7–82.7)	47.1 (22.9–72.2)
Sensitivity (%)	33,3 (0.8–90.6)	63.2 (38.4–83.7)	59.1 (36.4–79.3)
Specificity (%)	96.3 (81.0–99.9)	99.1 (70.3–100.0)	98.8 (61.6–100.0)
LR+	9.0 (0.99–69.27)	70.1 (2.25–2839.2)	47.8 (1.67–1938.4)
LR–	0.69 (0.21–1.0)	0.37 (0.19–0.61)	0.41 (0.23–0.70)
DOR	13.0	188.6	115.5

[a] Prevalence in the target population of patients with clinical suspicion of HVA.
[b] All proportions are expressed as percentages together with 95 % confidence interval
BAT bee – BAT performed with honeybee venom, BAT wasp – BAT performed with wasp venom, BAT total – BAT performed with BŰHLMANN CAST Allergens (irrespective of the type of allergen: bee venom or wasp venom), PPV – Predictive value of +ve test (post-test likelihood of disease), NPV – Predictive values of -ve test (post-test likelihood of no disease), LR+ – Likelihood Ratio positive test, LR- – Likelihood Ratio negative test, DOR – Diagnostic Odds Ratio. The results may be affected by small sample size.

The result of the BAT was considered positive, when more than 10 % of basophils were activated (basophil reactivity ≥ 10 %).

Statistical analysis

All participants' data were statistically described, analyzed for between-group differences, and summarized as means with respective standard deviations (SD) for continuous variables, or in contingency tables as counts or percentages. Agreement analysis was used to evaluate the degree of agreement and disagreement between the tested BAT method and the reference ELISA method over categories in 2x2 tables. Chi-square tests were used to test the agreement, disagreement and significant differences. Strength of agreement was expressed using the unweighted Cohen's kappa which measures the agreement between two sets of ratings discounting any element of agreement expected to have arisen by chance. A kappa above 0.8 indicates a very good agreement beyond chance. The McNemar´s statistic was used to test for asymmetry in the distribution of samples (subjects) where the ratings disagreed. The diagnostic performance, accuracy, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the screening test were calculated. All estimates were presented along with the respective 95 % confidence intervals (95 % CI).

Statistical analyses were performed using StatsDirect 3.2.7 software (Stats Direct Ltd., Cheshire, UK). All reported P values were two-sided, and significance was set at $p < 0.05$.

Tab. 4. Diagnostic performance analysis of BAT depending on symptom severity.

		No reaction + LLR	SR Grade I + Grade II	SR Grade II + Grade IV
BAT bee	Prevalence[a] (%)[b]	0.62	16.1	25.0
	PPV (%)	2.44	69.2	50.0
	NPV (%)	99.6	97.9	81.2
	Sensitivity (%)	50.0	90.0	40.0
	Specificity (%)	87.5	92.3	86.7
	LR+	4.0	11.7	3.0
	LR-	0.57	0.11	0.69
	DOR	7.0	108.0	4.33
BAT wasp	Prevalence[a] (%)[b]	19.0	64.5	85.0
	PPV (%)	54.5	89.2	92.9
	NPV (%)	99.5	72.0	33.33
	Sensitivity (%)	98.4	82.5	76.5
	Specificity (%)	80.8	81.8	66.7
	LR+	5.11	4.54	2.29
	LR-	0.02	0.21	0.35
	DOR	252.0	21.21	6.5

[a] Prevalence in the target population of patients with clinical suspicion of HVA.
[b] All proportions are expressed as percentages together with 95% confidence interval
BAT bee – BAT performed with honeybee venom, BAT wasp – BAT performed with wasp venom, No reaction + LLR – the group of patients with no reaction and patients with LLR (33 patients – 28.9 %), SR Grade I + Grade II – group of patients experienced a grade I or grade II reaction (61 patients – 53.5 %), SR Grade III + Grade IV – group of patients experienced a grade III or grade IV reaction (20 patients – 17.5), PPV – Predictive value of +ve test (post-test likelihood of disease), NPV – Predictive values of -ve test (post-test likelihood of no disease), LR+ – Likelihood Ratio positive test, LR- – Likelihood Ratio negative test, DOR – Diagnostic Odds Ratio. The results in some categories may be affected by small sample size

Results

In total, 114 subjects with suspicion of Hymenoptera venom allergy were enrolled; 41 % (47 patients) were males and 59 % (67 patients) were females. The average age was 47.5 ± 14.66 years, median age was 50 years (range 6–72 years), lower quartile 35.5 and upper quartile 61.5 years.

Based on the results of patient´s history, SPT, measurement of sIgE to whole insect venoms and molecular allergy diagnostics among the study population, 10 patients (8.8 %) were found to be allergic to honeybee venom, 58 patients (50.8 %) were allergic to wasp venom, 5 patients (4.4 %) were allergic to both venoms and 41 patients (36 %) were not allergic to honeybee or wasp. Five patients (4.4 %) had elevated serum tryptase level (> 11.4 ng/mL).

The BAT was performed with two different allergens. In the first 84 patients (73.7 %) commercial venom extracts for prick testing Soluprick SQ, ALK-Abelló, Denmark were used; the sample of each patient was examined with both honeybee and wasp venom, both in 3 different concentrations. In the next 30 patients (26 %), BŰHLMANN CAST Allergens, Germany were used, the sample of each patient was examined with both honeybee and wasp venom, both in one concentration recommended by the manufacturer.

1. Agreement and disagreement analyses of the BAT performed with honeybee venom and the BAT performed with wasp venom (irrespective of the type of allergen: Soluprick or BŰHLMANN): In honeybee testing the general agreement in 87.7 % was observed and chance-adjusted agreement expressed as Cohen's kappa was 0.54 (95 % CI 0.33 to 0.75; $p < 0.0001$). Disagreement and its asymmetry was not significant ($p = 0.109$).In wasp testing the general agreement was in 81.58 %, Cohen's kappa of 0.63 (95 % CI 0.48 to 0.77; $p < 0.0001$). No significant disagreement was observed ($p = 0.827$). Diagnostic performance analysis of the BAT is summarized in Table 1.
2. Agreement and disagreement analyses of the BAT performed with Soluprick SQ allergens that were used in 84 patients (73.7 %): In honeybee testing, the general agreement was observed in 86.9 % and Cohen's kappa was 0.56 (95 % CI 0.35 to 0.79; $p < 0.0001$). Here, a significant asymmetric disagreement ($p = 0.0348$), with a higher proportion of false positives (9) than false negatives was identified (2). These discordant proportions, however, were low in absolute values (in favor of high proportions of concordant ratings), so generalizability of the findings of disagreement beyond the sample is limited. In wasp testing the general agreement was observed in 83.33 % and Cohen's kappa was 0.66 (95 % CI 0.50 to 0.82; $p < 0.0001$). Disagreement was not significant ($p = 0.109$). Diagnostic performance analysis of the BAT performed with Soluprick SQ allergens are summarized in Table 2.
3. Agreement and disagreement analyses of the BAT performed with BŰHLMANN CAST Allergens that were used in 30 patients (26 %). The results may be affected by the small sample size. In honeybee testing we observed the general agreement in 90 % and Cohen's kappa was 0.35 (95 % CI –0.22 to 0.92; $p = 0.0255$). Disagreement was not significant $p = 0.564$. In

wasp testing the general agreement was observed in 76.97 % with Cohen's kappa of 0.56 (95 % CI 0.29 to 0.81; p = 0.0003). Disagreement with significant asymmetry (p = 0.0082) was detected with higher proportion of false negatives (7) than false positives (0). These proportions were low in absolute values, which does not allow drawing any conclusions that extend beyond the sample studied. Diagnostic performance analysis of the BAT performed with BŰHLMANN CAST Allergens are summarized in Table 3.

4. Diagnostic performance analysis of the BAT based on severity of symptoms. Eighty-one patients (71 %) had a documented history of SR after hymenoptera: 38 of them experienced a grade I reaction (33.3 % of all patients), 23 of them experienced a grade II reaction (20 % of all patients), 14 of them experienced a grade III reaction (12 % of all patients), and 6 of them experienced a grade IV reaction (6 % of all patients). Twenty-four patients (21 %) had a large local reaction and 9 patients (7.9 %) had no reaction. Diagnostic performance analyses of the BAT depending on severity of symptoms are summarized in Table 4.

Discussion

The basophil activation is emerging as a reliable and robust *in vitro* biomarker of allergy reactions occurring *in vivo* (10). In the past, testing the basophil response to allergen was focused on the measurement of mediators released by cells in the supernatant *in vitro*. This testing has largely been replaced by the BAT after the discovery of the up-regulation of CD63 during basophil activation in 1991 (16, 20).

Activation marker CD63 is a membrane protein localized in the same secretory lysosomal granule that contains histamine; therefore, histamine release and up-regulation of CD63 correlate well during activation of basophils. Where histamine is thought to be released by both piecemeal degranulation and anaphylactic degranulation, CD63 is a precise marker of anaphylactic degranulation through regulated exocytosis after allergen-mediated activation of basophils as well as mast cells. CD203c is frequently measured in addition to CD63 and appears to co-express with CD63 even though the pathways for up-regulation differ. CD203c highly reflects piecemeal degranulation (16, 22). The BAT uses the flow cytometry to measure the expression of these activation markers.

There are two common measures of basophil activity: basophil reactivity and basophil sensitivity. Basophil reactivity refers to the number of basophils that respond to a given stimulus and basophil sensitivity to the allergen concentration at which half of all reactive basophil respond (16).

The BAT is essentially an assay with superior specificity compared to any other allergy testing. If appropriately used, it can provide a valuable clinical asset in different areas of allergy diagnostics (23, 24, 25). Measurement and comparison of basophil sensitivity during the allergen immunotherapy can improve treatment response monitoring (10, 15). A successful VIT, which necessarily induces long term tolerance, decreases BAT sensitivity, without changing the reactivity (5).

In the present study, the culprit allergen was established based on the patient´s history, skin prick tests and determination of sIgE to the whole insect venoms and their individual allergens (rApi m 1, rVes v 1, rVes v 5) as well as sIgE to MUXF3 by the molecular allergy diagnostics. The sensitivity of skin prick test alone is around 64 %. The sensitivity of sIgE detection to yellow jacket venom for yellow jacket venom-allergic patients ranges between 83 % and 97 %. The sensitivity of sIgE detection to honeybee venom for honeybee venom- allergic patients ranges between 90 % and 100 %. Diagnostic sensitivity of a combination of two commercially available yellow jacket venom allergens Ves v 1 and Ves v 5 ranges between 92 % and 100 %. The first commercially available honeybee venom allergen Api m 1 yielded a diagnostic sensitivity of 58 % to 97 % (5). The combination of these tests improves the diagnostic precision in HVA and the identification of the appropriate venom for VIT.

The challenge testing was not performed. However, challenge testing is the gold standard for diagnosis of food or drug-induced anaphylaxis. A sting challenge with a live insect should not be used in patients with HVA who are not desensitized, because of the high risk of anaphylaxis. The allergen dose cannot be incrementally increased. Moreover, such sting challenge tests are not helpful in patients with a history of prior anaphylaxis, because the absence of early systemic symptoms does not exclude the possibility of a later systemic reaction (4, 8). Current recommendations advocate a sting challenge under an ongoing VIT only. A controlled sting challenge is considered to be the gold standard to evaluate the clinical response of the patient, allowing a reliable conclusion about the success of VIT (26). Despite maximum safety precautions and standby of emergency management, there is a potential of severe anaphylaxis, therefore sting challenge with a live insect is not perform routinely (25).

Although the BAT is not part of the routine diagnostics of venom allergy in all patients, it is well established and can be used in cases of unclear or negative skin and sIgE test results or when clinical history and diagnosis are contradictory. Studies demonstrated that BAT is able to detect sensitization in 80 % of venom-allergic patients with negative sIgE and in 60 % of patients that additionally exhibit negative intradermal skin tests (5). A study of the paediatric population revealed that the BAT allows for the identification of the culprit insect with higher specificity than the IgE reactivity test (27). The BAT has been suggested to be helpful in distinguishing a real double sensitization and cross-reactivity due to sequence homology among venom proteins. In addition, the BAT can be helpful in patients with systemic masocytosis or patients with high serum tryptase levels. In these patients total IgE and sIgE levels can be lover than in the general population. In general, these sIgE levels are below the traditional cutoff value, in which case there is difficulty in diagnosing HVA (28).

The diagnostic sensitivity of the BAT with insect venoms referred to the history was found to be 85–100 % and the diagnostic specificity was 83–100 % (15, 16). The sensitivity for the BAT measuring CD63 expression is reported as 89 % and 97 % for CD203c expression, but the use of the CD63 is more widespread (15, 16). In the present study, both activation markers (CD203c,

CD63) were measured to improve the sensitivity of BAT; CD203c was also used for gating the basophil population (10, 16).

Allergen source is one of critical factors in both clinical and research applications of the BAT. Allergen stimulants range from crude extracts to recombinant or purified single allergen sources. Standardized allergen reagents are recommended when comparing performance data from different laboratories and when performing tests over time. The availability of recombinant allergens for the BAT may be limited but they have the greatest stability and consistency compared to crude allergen or extracts and can help improve diagnostic accuracy (15, 16, 20).

The present study found the sensitivity of the BAT to be 80.8 % and the specificity to be 87.8 %. The prevalence was 64.0 %, the PPV was 92.2 % and the NPV was 72.0 %. The obtained sensitivity (80.8 %) was, in general, lower than previously reported values (85–100 %); the obtained specificity (87.8 %) confirmed literary data (15, 16, 17). The sensitivity, specificity, prevalence, PPV, and NPV obtained in the BAT with commercial venom extracts for SPT Soluprick SQ were 90.2 %, 84.9 %, 64.7 %, 90.2 %, 84.8 %, respectively. The sensitivity, specificity, prevalence, PPV, and NPV obtained in the BAT with BŰHLMANN CAST Allergens were 59.1 %, 98.8 %, 73.1 %, 99.2 %, 47.1 %, respectively.

The resulting value of BAT sensitivity was affected by the result of sensitivity of the BAT performed with BŰHLMANN CAST Allergens, which was much lower (51.9 %) than the sensitivity of the BAT performed with commercial venom extras for SPT Soluprick SQ (90.2 %), although BŰHLMANN CAST Allergens were optimized for *in vitro* use in the cellular allergy assays. The low sensitivity of the BAT with BŰHLMANN CAST Allergens may be attributable to the use of only one concentration of allergen recommended by manufacturer (11.5 ng/mL) whereas 3 different concentrations of Soluprick SQ Allergens (10, 100 and 300 µg) were used. Moreover, the results of the BAT with BŰHLMANN CAST Allergens could also be a result of the small sample size.

On the contrary, the specificity was higher for the BAT with BŰHLMANN CAST Allergens (98.8 %). The lower sensitivity of the BAT with Soluprick SQ Allergens (84.9 %) may have been caused by the use of allergens for SPT instead of allergens for intradermal tests (IDT), which are commonly used for the BAT (10). The allergen extracts for SPT are aqueous solutions of proteins combined with 50 % glycerol, which acts as a preservative. The solutions are, therefore, quite viscous. Glycerol is not included in IDT extracts as it may trigger false positive results during the intradermal testing. Included components and physical properties of SPT extracts may affect results of BAT specificity. Therefore, the allergen extracts for IDT are more respectable for BAT than allergen extracts for SPT (10, 29). In our study, we did not have the option to use allergen extracts for IDT because they are not available in Slovakia.

We found that the sensitivity of the BAT with wasp venom was higher (82.5 %) than the BAT with honeybee venom (73.3 %); conversely, the BAT with honeybee venom demonstrated higher specificity (89.9 %) than the BAT performed with wasp venom (80.4 %).

Our study did not confirm any association between the symptom severity and the sensitivity and specificity of BAT. This study is limited by the small number of cases in some categories according to severity of symptoms.

Our results confirmed that the BAT is a respectable method used in controversial cases of HVA reflecting high specificity and lower sensitivity. Therefore, the BAT is a confirmation test used for identifying the appropriate venom for VIT. For screening, the tests with high sensitivity are acceptable, such as skin tests and detection of sIgE. The sensitivity can be further increased by combining of these tests (10). In our study, we demonstrated the high specificity of BŰHLMANN CAST Allergens that are optimized for *in vitro* use in the cellular allergy assays. The sensitivity of the test with BŰHLMANN CAST Allergens may be increased by using different concentrations of allergens.

Conclusions

The BAT using flow cytometry can be helpful in the identification of the appropriate venom for VIT when used in combination with other well-established tests. Also, BAT could be recommended as a confirmatory test in the identification of the appropriate venom for VIT. Allergen source is one of critical factors in diagnostic reliability of the BAT, in our study we demonstrated the high specificity of BŰHLMANN CAST Allergens that are also available in Slovakia.

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