



DOUBLE POSITIVITY IN INSECT VENOM ALLERGY - DIAGNOSTIC APPROACH WITH BASOPHIL ACTIVATION TEST

O. Hausmann⁺, Th. Gentinetta⁺, M. Schneider^{*}, W. Pichler⁺, A. Helbling[#]

+ Department of Rheumatology, Clinical Immunology and Allergology Inselspital, Bern,

* Buhmann Laboratories Schoenenbuch, # SpitalNetzBern Zieglerspital, Div. of Allergology, Bern

Introduction

- Insect venom allergy is potentially life-threatening and venom immunotherapy a successful preventive treatment
- standard diagnostic work-up (skin tests, serology) irrespective of a clear-cut history reveals double-positivity (DP) to honey bee and wasp venom in up to 40% of patients. DP can be due to:
 - *Cross-reactive carbohydrate determinants (CCDs)* as part of glycosylated proteins of insect or plant origin (→ mostly lacking clinical relevance)
 - *partial peptide sequence identity* of venom proteins (→ probably clinically relevant)
 - *true double sensitization* to both venoms (→ requires treatment with both wasp & honey bee venoms)
- The CAP inhibition techniques did not prove to be helpful in differentiation between those groups

In this study, we examined the usefulness of a basophil activation test (BAT) to differentiate between crossreactivity and true allergy in comparison to the above mentioned CAP-based techniques.

Methods

9 insect venom allergic patients (grade III-IV)

Insect: 6 wasp 3 not identified (wasp suggestive)

double positive in skin test (i/d) and/or sIgE

11 healthy stung controls

16 bee venom allergic patients **ROC analysis**

	Flow2CAST	CAP Inhibition
Blood sample	Whole blood EDTA	Serum
Prestimulation	+/- IL3	4 vials for each venom: - <u>no</u> inhibition (reg. CAP) - <u>homonymous</u> inh. (4h) as positive control - <u>heteronymous</u> inh. (4h) - <u>baseline</u> (buffer)
Selection marker	CCR3	
Activation marker	CD63	
Positive Control	anti-FcεRI fMLP	
Allergen	Bee venom 2 – 285 ng/ml	0-20 % Inh. negative (DP) 21-65 % Inh. intermediate >65 % Inh. positive (CR)

Results

	PATIENT 1	PATIENT 2	PATIENT 3	PATIENT 4	PATIENT 5	PATIENT 6	PATIENT 7	PATIENT 8	PATIENT 9
History Season	wasp october	n.id. may	wasp september (end)	wasp june	wasp august	hornet june	wasp july (end)	n.id. summer	n.id. june
Skin test sIgE [kU/l]	BV 10 ⁻⁴ WV 10 ⁻⁴ BV 3.6 WV 1.2	BV 10 ⁻⁴ WV 10 ⁻⁶ BV 6.8 WV 3.4	BV 10 ⁻⁴ WV 10 ⁻⁶ BV 0.96 WV 1.23	BV 10 ⁻⁴ WV 10 ⁻⁶ BV 11.3 WV 13.4	BV 10 ⁻⁶ WV 10 ⁻⁴ BV 5.34 WV 4.37	BV 10 ⁻⁴ WV 10 ⁻⁶ BV 1.86 WV 0.47	BV 10 ⁻⁴ WV 10 ⁻⁴ BV 14.2 WV 2.44	BV neg. WV 10 ⁻⁴ BV 0.89 WV 0.58	BV 10 ⁻⁴ WV 10 ⁻⁶ BV 49.3 WV 6.75
total IgE [kU/l]	30.6	39.0	71.0	147.0	n.d.	n.d.	235.0	43.8	172
CAP-Inh.	BV 0/65%	BV / WV 0/0%	BV / WV 0/13%	BV / WV 5/0%	BV(partial) / WV 41/0%	BV(partial) / WV 59/0%	BV / WV(partial) 5/23%	BV / WV(partial) 3/40%	BV / WV(partial) 3/20%
Bromelain	1,69 kU/l KI.2	<0.35 kU/l neg.	<0.35 kU/l neg.	1,23 kU/l KI.2	<0.35 kU/l neg.	<0.35 kU/l neg.	0.38 kU/l KI.1	<0.35 kU/l neg.	0,69 kU/l
Result	B / DP	DP	DP	DP	DP	DP / W	DP	DP	DP
BAT with IL-3	DP	DP	W	DP	DP	W	B ?	W	DP
BAT w/o IL-3	DP	W	W ?	DP	DP	W ?	B	W	DP
	DP	W / DP	W	DP	DP	W	B	W	DP

BV: bee venom; WV: wasp venom; n.id.: not identified; n.d.: not done

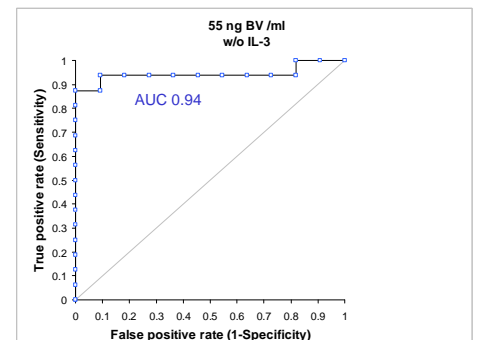
Discussion

- BAT was useful in the diagnosis in almost half of the patients (4/9) that correlated well with the history and the skin test results
- CAP inhibition assays often show equivocal or intermediate results and are therefore not really helpful in the clinical routine
- The use of CCDs of plant origin (bromelain-albumin complex) used in CAP inhibition may enhance test performance
- CCD positivity does not exclude double positivity in the BAT assay
- The role of IL3 addition remains to be clarified as it seems to be mainly enhancing low-affinity IgE with uncertain clinical relevance

Conclusion

BAT is a useful *in vitro* tool in identifying the culprit insect venom in case of double positivity to both wasp and honey bee venom.

ROC analysis



Bee venom	CD63 upreg.	Sensitivity	Specificity
with IL-3	5,4 – 11,3 %	87,5 – 93,8 %	90,9 – 100 %
w/o IL-3	2,3 – 3,4 %	76,9 – 92,3 %	90,9 – 100 %