

Towards a clinical validation of the BÜHLMANN Flow2 CAST® basophil activation test for drug and protein allergy

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Introduction

Since several years Basophil activation tests (BAT) are successfully used for the allergy diagnosis in cases where common approaches as specific IgE (sIgE) or histamine release tests failed. During this time the CD63 surface marker established itself as the most popular activation marker. Due to heterogeneous and sometimes complex protocols BAT still plays only a marginal role in allergy diagnosis.

Results Drug allergens (Data from groups 2 and 5)

Controls (n=11) were tested for the following allergens (in 3 concentrations): PenG, PenV, PPL, MDM, Ampicillin, Amoxicillin and Cefuroxim. Patients (n=21 in group 2 and n=16 in group 5) were tested at least for culprit, MDM and PPL. A result was set positive when one of the allergens gave a stimulation of above 5% CD63 (SI>2).

Group	PenG		PenV		PPL		MDM		Amox		Amp		Cef		Total		Spec
	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	
Group 2																	
Controls	0	11	0	11	0	11	0	11	0	11	0	11	2	9	2	9	Spec 82%
Patients	7	8	6	8	1	19	4	16	0	11	0	2	6	4	10	11	Sens 48%
Group 5																	
Patients	1	15	3	13	5	11	2	14	1	15	1	15	1	0	8	8	Sens 50%
Group 2 and 5																	
Controls	0	11	0	11	0	11	0	11	0	11	0	11	2	9	2	9	Spec 82%
Patients	8	23	9	21	6	30	6	30	1	26	1	17	7	4	18	19	Sens 49%

Table 1: Number of patients and controls negative or positive for testing with individual allergens. Results of the individual groups and the combination of both groups.

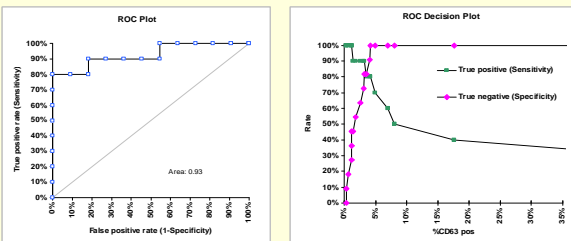


Fig 1: With the data of 10 Cefuroxim patients and 11 controls a ROC curve only with Cefuroxim (c=570 µg/ml) results was generated. Using a cut-off of 5% CD63 activation resulting in 70% sensitivity and 100% specificity for Cefuroxim.

Conclusion Drugs

The observed sensitivity for antibiotics of 48 to 50% and the specificity of 82% are highly comparable to other already published data.

Ref: Blanca M. et al.: Update on the evaluation of hypersensitivity reactions to betalactams (2009) Allergy 64:183-193.

Food hypersensitivity in children (Data from group 6)

From six children with proven food hypersensitivity (challenge) four showed a clear positive result in the Flow2 CAST® assay. A result was set positive when one of the allergen dilution gave a stimulation of above 15% CD63.

Age	Sex	Anamnesis	Spec. IgE				Total IgE	Flow2 CAST Results			
			Peanut	Sesame	Soy	Wheat		Peanut	Sesame	Soy	Wheat
9	m	Sesame/Soy	IV	IV	III	II	2537	pos	neg	neg	-
4	f	Peanut	I	0	0	0	out	neg	neg	-	-
10	m	Soy	IV	IV	III	III	5000	pos	neg	neg	neg
8	f	Wheat/Soy	III	IV	III	II	1301	pos	pos	-	pos
15	m	Peanut	III	II	I	I	1308	pos	neg	-	neg
3	m	Wheat	II	II	0	0	294	neg	-	neg	-

Table 2: Comparison of specific IgE measurement (RAST classes) with Flow2 CAST® results. Allergen concentration during stimulation with diluted whole blood: c1= 23 ng/ml, c2=95 ng/ml.

Conclusion Food hypersensitivity

Compared to specific IgE less false positive results were observed, thus the specificity of the test seems to be higher.

Conclusion

Clinical studies in different European clinical centers with the Flow2 CAST® kit showed the same good sensitivities and specificities as published earlier with other BAT methods.

The test is easy to handle because of its whole blood protocol and easy to evaluate compared with other BAT methods due to the use of only two markers: The stable expressed surface marker CCR3 for the easy selection of the Basophilic cells and CD63 as activation marker.

These evaluations confirm that this test has the potential to be a very reliable tool for routine application in cellular allergy diagnosis for drugs, hymenoptera venom and food allergy.

Methods

Five different European clinical centers in Wrocław, PL (group 4), Munich, DE (group 2), Berne, CH (group 3), Rome, IT (group 5) and London, UK (group 6) evaluated the commercially available Flow2 CAST® (CE-labeled IVD test) based on stimulation of whole blood measuring CD63 activation on basophilic cells selected by the high specific surface marker CCR3 using flow cytometry.

In total 152 patients and controls were evaluated. Two clinical centers tested patients allergic to antibiotics, one group tested patients allergic to bee venom and house dust mite (HDM)/grass pollen, and one group tested children allergic to food (Peanut, Sesame, Soy, Wheat).

Results Bee Venom (Data from group 3)

Patients with allergy to bee venom (n=17) and stung controls (Flow2 CAST® n=11, Flow-CAST® n=38) were tested for bee venom allergen in the concentration range from 0.46 to 2270 ng/ml. c1= 2270, c2=1425, c3=285, c4=57, c5=11.4, c6=2.3 and c7=0.46 ng/ml.

Bee venom conc in stimulation	Area under curve	cut off	Sens	Spec	Likelihood ratio (+)	Likelihood ratio (-)	
ng/ml		%CD63+	%	%			
c3	285	0.96	15	93.8	90.9	10.31	0.07
c4	57	0.94	10	93.8	90.9	10.31	0.07
c5	11.4	0.96	10	93.8	90.9	10.31	0.07
c6	2.28	0.91	10	87.5	90.9	9.63	0.14
c5	25	0.97	10	94.1	92.1	11.92	0.06

Table 3: Results from ROC analysis for 4 different bee-venom concentrations in Flow2 CAST® in comparison to ROC analysis of one Flow-CAST® concentration.

Conclusion Bee Venom

The observed sensitivity of 94% and specificity of 91% are highly comparable with the results gained with the more laborious Flow-CAST® assay (Sens: 94%, Spec: 92%)*. Best analytical discrimination was observed at an allergen concentration of 11.4 ng/ml.

* Ref: Scherer K. et al.: Cellular in vitro Assays in the Diagnosis of Hymenoptera Venom Allergy (2008) Int Arch Allergy Immunol 146: 122-132.

Results HDM and Grass pollen (Data from Group 4)

Patients with allergy to HDM (n=16) and grass pollen (n=12) were tested with two different concentrations of HDM (c1= 250 ng/ml, c2= 25 ng/ml) and 6-grass mix (c1=25 ng/ml, c2=2.5 ng/ml). Together with the results gained from controls (n=19) ROC curves were calculated to find the best discrimination allergen concentration.

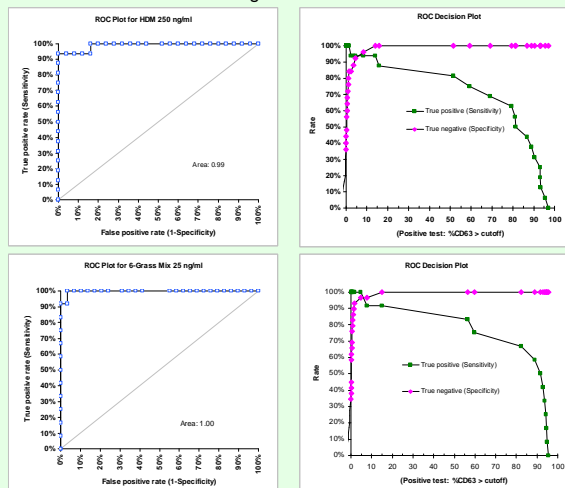


Fig 2: ROC curve for HDM (D1) 250 ng/ml give optimal cut off at 15%. For 6-grass mix a cut-off of 15% is best for 25 ng/ml.

Conclusion HDM/Grass pollen

Best analytical discrimination was observed at 250 ng/ml for HDM and 25 ng/ml for grass-mix with a sensitivity of 94% and 92% and a specificity of 100% (n=19), respectively.

These data correspond to earlier published data.

Ref: Sanz ML. et al.: Allergen-induced basophil activation: CD63 cell expression detected by flow cytometry in patients allergic to *D. pteronyssinus* and *L. perenne* (2001) Clin Exp Allergy 31:1007-13.

Take home message

- These evaluations show the capability of Flow2 CAST® to be a reliable tool in difficult cases of allergy diagnosis.
- The simple and fast (results within 1 hour) whole blood based protocol has the potential to become a new standard in Basophil Activation Testing using Flow Cytometry.