Correlation of sting challenge outcome and change in EC50 in basophil activation test (BAT) in bee venom allergic patients after 2-5 years of venom immunotherapy

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Background

Efficacy of venom immuntherapy (VIT)

No in-vitro surrogate marker for success of VIT

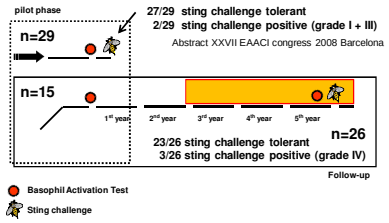
→ Sting challenge recommended with / without systemic reaction (SR)

	SR on sting challenge after VIT (5yr.)	
	Honey Bee	Vespid
Gillman 1980	22% (4/18)	nd
Hoffman 1981	20% (5/25)	nd
Golden 1981	nd	3% (4/143)
Müller 1992	23% (34/148)	9% (5/57)
Rüeff 2013	11% (10/90)	4% (12/267)

Hypothesis

- **Basophil activation test (BAT) is able to identify those bee venom allergic subjects who are not protected under standard VIT (positive sting challenge, SC)**

Project Overview



27/29 sting challenge tolerant
2/29 sting challenge positive (grade I + III)
Abstract XXVII EAACI congress 2008 Barcelona

23/26 sting challenge tolerant
3/26 sting challenge positive (grade IV)

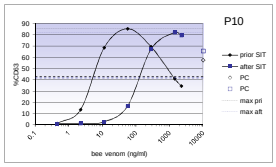
Study Details

- **Bee venom allergic patients**
- **3.5 logs of allergen concentration**

		conc. after recost in 250 µl	conc. in FK-CCR dilution 1:4.4
Bee-venom	c1	10000 ng/ml	2273 ng/ml
BAG2-I1	c2	6250 ng/ml	1420 ng/ml
	c3	1250 ng/ml	284 ng/ml
	c4	250 ng/ml	56.8 ng/ml
	c5	50 ng/ml	11.4 ng/ml
	c6	10 ng/ml	2.27 ng/ml
	c7	2 ng/ml	0.45 ng/ml

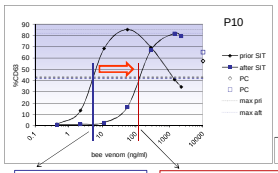
- **Study length of 7 years (2007-2013)**
- **Flow CAST® Test conditions:**
  whole blood, IL-3 containing stimulation buffer, 15 min. incubation time, count ≥ 500 basophils.

Evaluation template



ABP = "reactivity" activated basophil population
Maximal number of activated basophils (in % of total) upregulating CD63 after stimulation with either bee venom or anti-FcεRI (PC)

EC50 = "sensitivity"
Bee venom concentration resulting in half-maximal ABP

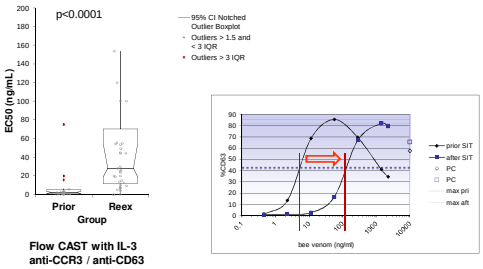


EC50 = "sensitivity"
Bee venom concentration resulting in half-maximal ABP

$$\text{EC50-Ratio} = \frac{EC50\ (after\ VIT)}{EC50\ (prior\ VIT)} = \frac{120\ ng/ml}{5\ ng/ml} = 24$$

EC50 prior VIT: 5 ng/ml **EC50 after VIT: 120 ng/ml**

Results



Flow CAST with IL-3 anti-CCR3 / anti-CD63

Statistics

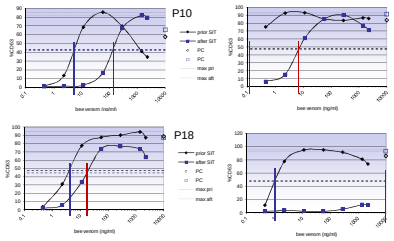
Patient	Sting Challenge	EC50 Ratio
10	tolerant	24
11	tolerant	17.5
16	tolerant	2
18	tolerant	5
31	tolerant	2500
33	tolerant	0.01
34	tolerant	0.875
35	reactive	0.7
37	tolerant	6.7
41	tolerant	8
45	tolerant	5.3
57	tolerant	18.8
60	tolerant	12.9
61	tolerant	4
62	tolerant	1
64	tolerant	44.4
65	tolerant	55.6
66	tolerant	13.3
67	reactive	0.02
69	tolerant	5.7
70	tolerant	1.6
71	tolerant	1032
73	tolerant	5
75	reactive	1.2
76	tolerant	66.7

EC50 Ratio	Sting Challenge reactive	tolerant	Total
Positive test < 1.6	3	3	6
Negative test ≥ 1.6	0	20	20
Total	3	23	26

Sensitivity	100%
Specificity	87%
PPV	50%
NPV	100%

Results detailed

Reduced bee venom sensitivity in 20/23 pat.



No reduction in BAT sensitivity in SC reactors (3/26)

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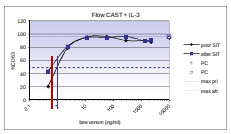

	prior SIT	after SIT	Ratio
EC50 (ng/ml)	0.9	0.6	0.7

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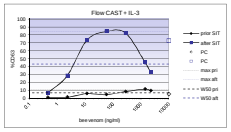

	prior SIT	after SIT	Ratio
EC50 (ng/ml)	200	4	0.02

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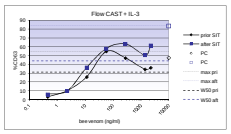
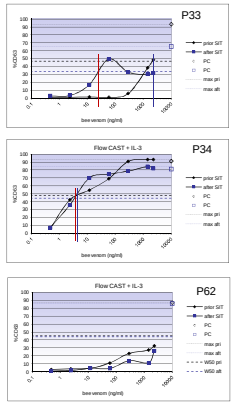


	prior SIT	after SIT	Ratio
EC50 (ng/ml)	15	18	1.2

discrepant results in SC tolerant pat. (3/26)



False-positive BAT?
False-negative SC?

Conclusions

- **Correlation of EC50 ratio of >1.6** with tolerated sting challenge in **20/26 patients**
- 3/26 (11.5%) of sting challenge tolerant patients misstratified (**BAT specificity 87%**)
- **No sting challenge reactive reactive patient was missed (BAT sens. 100%)**
- Relatively low rate of sting challenge reactors **(3/26 pat., 11.5%)**
- BAT with bee venom conc.range (EC50) may be a valuable predictive tool for the success of VIT, especially in situations when sting challenges with living insects are not feasible

→ identification of **unprotected VIT patients**

References

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