

Clinical Communications

Possible utility of basophil activation test in dual honeybee and vespid sensitization

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Clinical Implications

- The basophil activation test may be a useful tool to exclude clinically irrelevant sensitization in otherwise double-sensitized allergic patients. Implementation as a complementary diagnostic tool may reduce the frequency of unnecessary double venom immunotherapies.

TO THE EDITOR:

The identification of the appropriate venom for hymenoptera venom immunotherapy is often hampered by a high frequency of double sensitizations (DS) to honeybee and vespid venom. Depending on the method used, up to 64% of insect venom—allergic patients show double-positivity.^{1,2} Three main reasons for DS are currently known: (1) true DS to different bee and vespid venom allergens, (2) DS caused by cross-reactive specific IgE (sIgE) antibodies that recognize similar protein epitopes of the hyaluronidase, dipeptidylpeptidase, or vitellogenin of both venoms, and (3) the presence of sIgE to cross-reactive carbohydrate determinants in bee and vespid venom allergens.³

The introduction of component-resolved diagnosis was considered to significantly improve the diagnosis of insect venom allergy. Although the use of rVes-v-5 combined with rVes-v-1 was sufficient for a correct diagnosis of vespid venom allergy,⁴

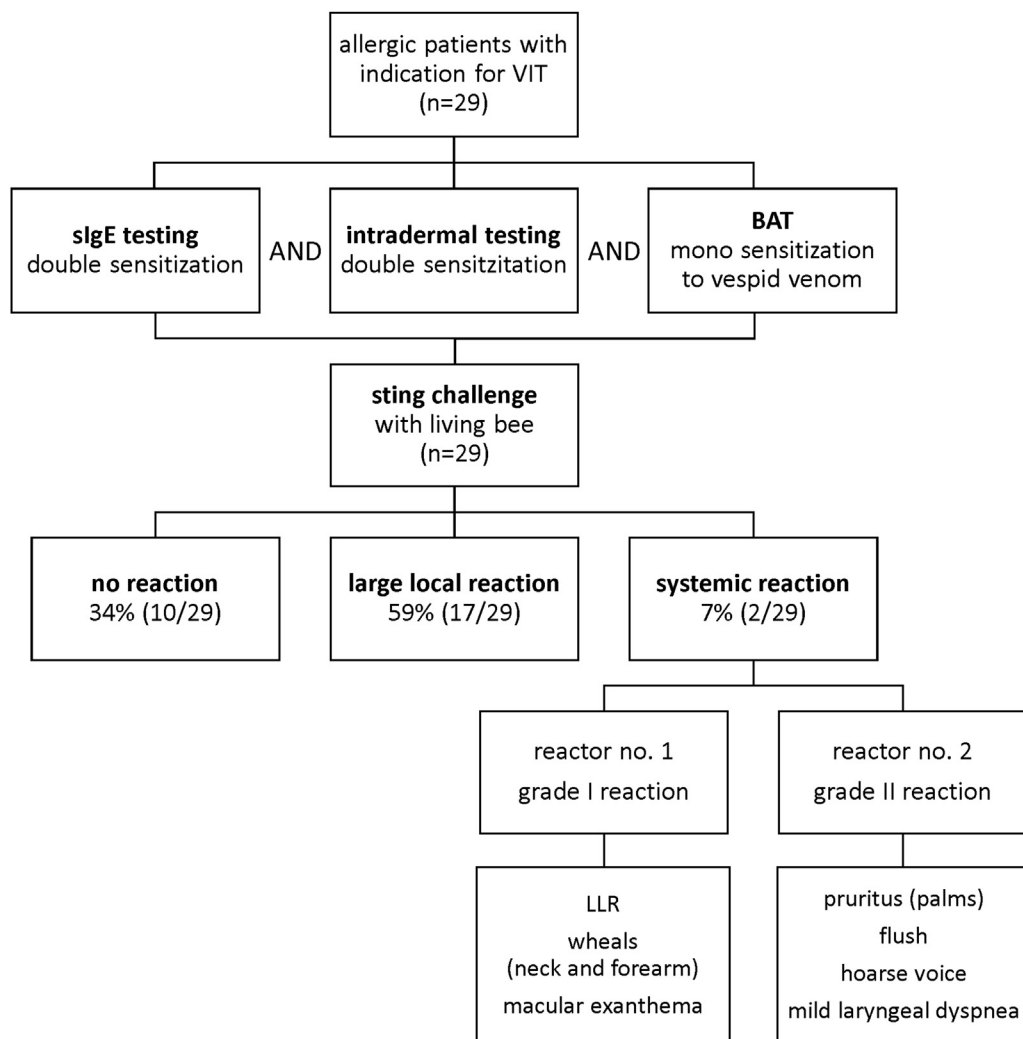


FIGURE 1. Characteristics of included patients and outcome of sting challenges. VIT, Venom immunotherapy.

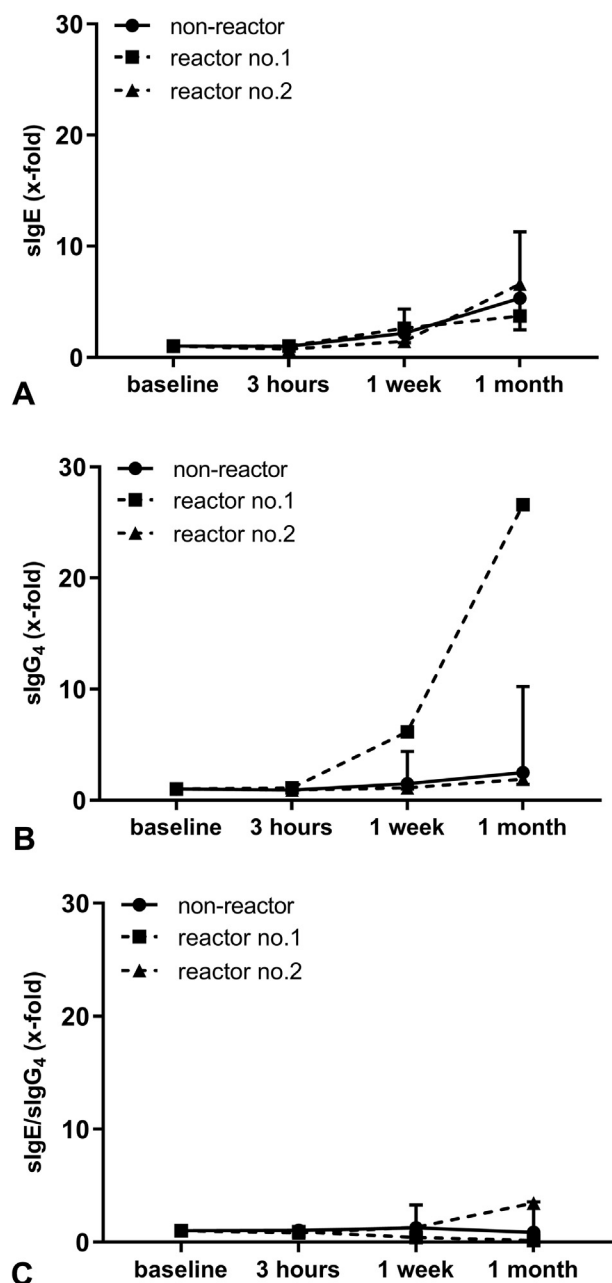


FIGURE 2. Shift of sIgE to BV (A), sIgG₄ (B), and sIgE/sIgG₄ ratio (C) after bee stings in nonreactors and reactors. Data are expressed as medians (interquartile ranges) for nonreactors and single data points for reactors.

the usefulness of the component-resolved diagnosis in bee venom (BV)-allergic patients in the present form was questionable due to the low sensitivity of the whole panel of BV allergens and the fact that double-positivity could not be sufficiently worked up.⁵ IgE inhibition assays are useful in DS, especially if heterologous inhibition is high and the primary sensitization can be identified. However, if only partial heterologous inhibition can be achieved, the clinical relevance of DS remains unclear.

The basophil activation test (BAT) is a functional test, where basophil activation occurs only if an allergen has the

ability to crosslink 2 identical FcεRI-bound IgE antibodies on the cell surface of basophils. Low-affinity IgE resolve faster from the Fcε receptor and is less likely cross-linked than high-affinity IgE.^{2,6} A lower frequency of double-positive test results to bee and vespid venom with the BAT was previously described.² However, BAT-negative results in double-positive patients have never been validated so far. Therefore, the aim of this study was to evaluate the negative predictive value of the BAT by performing sting challenges with the BAT-negative insect in otherwise double-positive venom-allergic patients.

Twenty-nine patients with a history of a systemic sting reaction (SSR) and indication for venom immunotherapy were included in the study. All patients showed DS to honeybee and vespid venom in sIgE testing (ImmunoCAP, Thermo Fisher Scientific, Waltham, Mass) and intradermal testing (ALK-Abelló, Hørsholm, Denmark), but monosensitization to vespid venom in the BAT (Bühlmann Laboratories, Schönenbuch, Switzerland) and underwent sting challenges with living honeybees (see Figure 1; also see Table E1 in this article's Online Repository at www.jaci-inpractice.org). Blood samples were collected at baseline before the sting challenge, as well as 3 hours, 1 week, and 4 weeks after the sting challenge. The study was approved by the ethics committee of the Medical University of Graz (approval no. 23-198 ex 10/11).

Most patients (27 of 29; 93%) tolerated the sting challenge without SSR; however, 2 patients (2 of 29; 7%) had systemic sting reactions: the first patient had a weak grade I reaction, and the second patient suffered from a mild grade II reaction according to Ring and Messmer. Notably, sting challenge caused large local reactions (LLRs) in 17 of 29 (59%) patients (see Figure 1).

Testing for statistical differences between nonreactors and reactors was hampered by the small number of reactors. However, no significant differences were found concerning demographic data, results of initial testing, or baseline characteristics (see Tables E1 and E2 in this article's Online Repository at www.jaci-inpractice.org).

After sting challenges, levels of sIgE to BV developed similarly in nonreactors and reactors, while sIgG₄ levels increased clearly after 1 month in 1 reactor (see Figure 2). The shifts in sIgE levels to BV components after bee stings also did not differ significantly (see Figure E1 in this article's Online Repository at www.jaci-inpractice.org). A booster effect of sIgE to BV caused by the sting was observed in 21 of 24 (88%) nonreactors. Furthermore, the sensitization pattern to BV components changed after sting challenges in more than half the patients over time. However, new sensitizations to BV allergens due to the sting challenge were unlikely, because in most patients sIgE to molecular allergens were detectable under the cutoff of 0.35 kU/L before (see Table E3 in this article's Online Repository at www.jaci-inpractice.org). This is in accordance with previous data, that sIgE increased in asymptotically sensitized subjects,^{7,8} but this was not an indicator for conversion into symptomatic sensitization.⁷ The BAT appeared to be a reliable tool to exclude moderate to severe SSR to BV in double-sensitized allergic patients. In both reactors, SSRs were mild in contrast to their initial sting reactions, both of which led to loss of consciousness. Furthermore, it can be assumed that all included patients had a genuine DS because all had double-positive intradermal tests and the vast majority had detectable sIgE to BV components.

Although serological sensitization to BV did not lead to SSR after sting challenges in most subjects, sensitization was not asymptomatic: 63% (17 of 27) of all subjects developed an LLR, which can be burdensome and last over several days. These results demonstrate that positive sIgE levels are often relevant for LLR in so-called asymptomatic subjects. It is well known that most patients with LLR display positive venom sIgE and/or skin test results⁹; however, the pathomechanism has not been sufficiently addressed. An IgE-mediated or cell-mediated cutaneous late-phase reaction may be responsible for the development of LLR; this could be the reason that the BAT result was negative in these subjects. In addition, clinically irrelevant low-affinity sIgE antibodies, which are less likely cross-linked on the cell surface of basophils, may explain negative BAT results.

However, there are some important limitations of the BAT. First, blood specimens should be analyzed within 2 days after collection and the rapid transport to a specialized laboratory may be difficult sometimes. Second, a subset of patients will be nonresponders and thus unsuitable for BAT evaluation. Third, optimizing and maintaining the BAT requires special technical skills because the assay is not automated and many (error-prone) manual steps have to be done. Therefore, only a few laboratories are able to perform a quality-controlled BAT.

Although not perfectly excluding any SSR, the BAT was a useful tool in detecting clinically irrelevant sensitization to BV in otherwise double-sensitized allergic patients. Implementation of the BAT as a complementary diagnostic tool may reduce the frequency of unnecessary double venom immunotherapies. However, larger studies are needed to confirm our findings.

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Conflicts of interest: The authors declare that they have no relevant conflicts of interest.

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ONLINE REPOSITORY

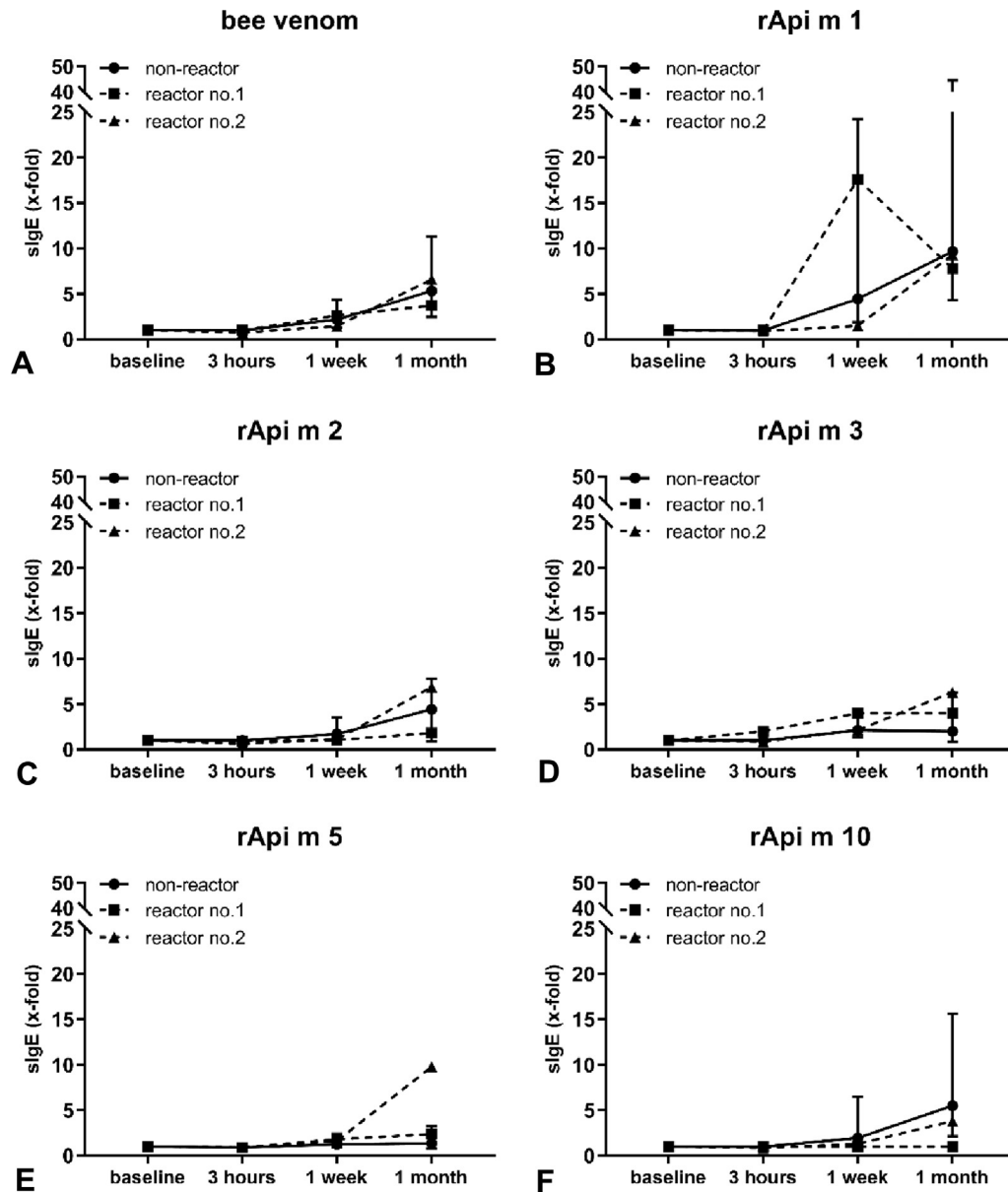


FIGURE E1. Shift of sIgE to BV (A), rApi m 1 (B), rApi m 2 (C), rApi m 3 (D), rApi m 5 (E), and rApi m 10 (F) after bee stings in nonreactors and reactors. Data are expressed as medians (interquartile ranges) for nonreactors and single data points for reactors.

TABLE E1. Demographic data and results of initial testing (baseline)*

Characteristic	Nonreactors (n = 27)	Reactor no. 1	Reactor no. 2
Age range (mean) (y)	19-73 (43)	49	41
Sex, n (%)			
Female	9 (33)	0 (0)	1 (100)
Male	18 (67)	1 (100)	0 (0)
Atopy, n (%)	20 (74)	1 (100)	1 (100)
Grade of SSR (index sting), n (%)			
I°	2 (7)	0 (0)	0 (0)
II°	16 (59)	0 (0)	0 (0)
III°	9 (33)	1 (100)	1 (100)
IV°	0 (0)	0 (0)	0 (0)
sIgE determination (kU/L)†			
BV	2.65 (0.78; 5.78)	1.14	2.73
Vespid venom	5.26 (2.26; 17.80)	3.12	15.10
Intradermal test (minimum concentration giving positive result) (µg/mL), n (%)			
BV			
0.01	1 (4)	0 (0)	0 (0)
0.1	8 (30)	0 (0)	1 (100)
1.0	18 (67)	1 (100)	0 (0)
Vespid venom			
0.01	8 (30)	0 (0)	1 (100)
0.1	15 (56)	1 (100)	0 (0)
1.0	4 (15)	0 (0)	0 (0)
BAT (maximum percentage of activated basophils)†			
BV	4.3 (2.6; 6.0)	1.7	1.9
Vespid venom	45.3 (18.3; 74.3)	34.5	46.5

*No statistically significant differences between nonreactors and the 2 reactors were detected.

†Median (lower and upper quartiles).

TABLE E2. Baseline results of total IgE, sIgE, and sIgG₄ determination, sIgE/sIgG₄ ratios, and tryptase levels

Immunological parameters	Nonreactors (n = 27), median (lower and upper quartiles)	Reactor no. 1	Reactor no. 2
Total IgE (kU/L)	127.0 (60.5; 301.0)	119.0	126.0
sIgE BV (kU/L)	2.65 (0.78; 5.78)	1.14	2.73
sIgE rApi m 1 (kU/L)	0.09 (0.04; 0.37)	0.05	0.10
sIgE rApi m 2 (kU/L)	0.13 (0.02; 0.47)	0.64	0.55
sIgE rApi m 3 (kU/L)	0.09 (0.04; 0.53)	0.01	0.21
sIgE rApi m 5 (kU/L)	1.89 (0.54; 6.41)	0.55	0.13
sIgE rApi m 10 (kU/L)	0.08 (0.03; 0.26)	0.02	0.20
sIgE VV (kU/L)	5.26 (2.26; 17.80)	3.12	15.10
sIgE rVes v 1 (kU/L)	0.60 (0.13; 5.45)	0.10	0.11
sIgE rVes v 5 (kU/L)	6.41 (2.26; 11.40)	9.36	13.10
sIgE CCD-MUXF3 (kU/L)	0.10 (0.02; 1.48)	0.13	1.63
sIgG ₄ BV (kU/L)	0.43 (0.14; 1.38)	0.19	0.10
sIgG ₄ rApi m 1 (kU/L)	0.11 (0.02; 0.40)	0.17	0.12
sIgG ₄ VV (kU/L)	2.29 (0.69; 8.01)	6.46	0.10
sIgG ₄ rVes v 5 (kU/L)	0.92 (0.32; 2.28)	11.60	0.06
sIgE/sIgG ₄ ratio BV	8.49 (1.84; 29.44)	6.00	27.30
sIgE/sIgG ₄ ratio VV	3.32 (1.14; 7.10)	0.48	151.00
Tryptase (µg/L)	4.80 (2.70; 6.80)	2.40	5.30

CCD, Cross-reactive carbohydrate determinant; VV, vespid venom.

TABLE E3. Sensitization profile of nonreactors and reactors at baseline, 3 h after sting challenge, 1 wk after sting challenge, and 1 mo after sting challenge

Patient ID	Time point	slgE bee	slgE rApi m 1	slgE rApi m 2	slgE rApi m 3	slgE rApi m 5	slgE rApi m 10	slgE wasp	slgE rVes v 1	slgE rVes v 5
1*	Baseline	5.77	0.02	2.41	0.54	1.22	0.11	5.26	1.06	10.10
	3 h	7.10	0.01	2.12	0.48	1.09	0.10	16.40	14.20	12.00
	1 wk	37.30	0.18	18.10	9.18	5.30	9.43	72.80	178.00	89.20
	1 mo	65.20	0.18	48.80	5.04	9.66	2.81	52.50	60.90	95.20
2	Baseline	0.56	0.08	0.11	0.05	0.82	0.00	2.26	1.75	0.44
	3 h	0.63	0.09	0.12	0.07	0.83	0.01	2.39	1.70	0.45
	1 wk									
	1 mo									
3	Baseline	2.52	0.26	0.02	0.57	2.11	0.38	11.60	6.14	11.40
	3 h	2.73	0.22	0.04	0.52	1.66	0.33	10.90	5.23	10.20
	1 wk	2.65	0.28	0.05	0.52	1.91	0.35	10.80	5.03	9.76
	1 mo	8.66	1.19	0.04	0.46	2.27	0.39	18.40	8.81	15.10
5*	Baseline	4.46	0.04	0.07	0.25	1.94	0.04	5.69	0.21	8.75
	3 h	5.64	0.06	0.12	0.26	1.65	0.05	6.87	0.24	9.07
	1 wk	12.40	4.46	0.88	0.99	2.90	0.12	6.69	0.27	8.80
	1 mo	52.30	17.50	3.41	1.05	3.58	0.22	18.20	0.55	12.70
6	Baseline	4.82	0.04	0.13	0.05	0.10	0.10	4.57	0.03	21.50
	3 h	3.77	0.03	0.11	0.05	0.07	0.08	4.09	0.02	16.80
	1 wk	56.50	1.13	1.98	2.63	2.29	0.67	12.50	0.08	23.40
	1 mo	65.70	1.31	6.59	1.62	1.91	1.39	14.30	0.18	20.80
7*	Baseline	2.65	0.33	0.40	1.51	2.12	0.38	9.37	5.45	0.38
	3 h	2.19	0.37	0.33	1.45	1.57	0.35	8.46	5.30	0.41
	1 wk	2.81	0.45	0.51	2.02	1.93	0.35	8.47	5.36	0.30
	1 mo	20.70	1.41	2.11	7.06	6.10	2.09	25.80	7.04	0.86
8	Baseline	0.57	0.06	0.83	0.00	0.94	0.00	4.51	0.32	11.00
	3 h	0.59	0.06	0.69	0.02	0.75	0.01	8.86	0.31	8.02
	1 wk	0.76	0.11	0.82	0.02	0.95	0.00	6.08	0.48	14.70
	1 mo	0.61	0.08	0.78	0.01	0.74	0.00	3.96	0.22	8.84
9*	Baseline	0.37	0.11	0.00	0.11	0.35	0.03	1.03	0.33	3.38
	3 h	0.29	0.09	0.04	0.11	0.31	0.03	2.54	0.37	2.32
	1 wk	21.40	19.40	1.37	5.53	1.07	0.71	1.04	0.39	2.90
	1 mo	20.90	18.20	1.34	2.32	0.81	0.66	5.02	3.06	3.39
10*,†	Baseline	1.14	0.05	0.64	0.01	0.55	0.02	3.12	0.10	9.36
	3 h	1.14	0.05	0.58	0.02	0.49	0.02	3.12	0.16	9.36
	1 wk	2.97	0.88	0.66	0.04	0.99	0.02	3.32	0.13	7.41
	1 mo	4.23	0.39	1.16	0.04	1.30	0.02	3.93	0.14	8.17
11*	Baseline	1.57	0.16	0.01	2.01	0.00	0.06	0.92	0.37	2.94
	3 h	1.44	0.04	0.03	1.87	0.03	0.06	0.89	0.34	2.99
	1 wk	3.51	0.33	0.03	5.76	0.03	0.08	0.92	0.36	2.71
	1 mo	18.30	9.53	0.37	2.48	1.34	0.69	6.86	0.42	2.93
12	Baseline	1.29	0.12	0.00	0.04	0.15	0.08	9.92	8.74	2.26
	3 h	1.28	0.13	0.01	0.05	0.13	0.07	9.82	8.18	2.31
	1 wk	1.90	0.37	0.01	0.09	0.14	0.10	10.50	7.84	2.30
	1 mo	23.30	9.81	0.02	0.73	0.51	1.66	9.69	8.79	2.19
13*	Baseline	3.76	0.06	2.25	0.50	0.30	0.26	4.12	0.60	5.93
	3 h	3.94	0.09	2.31	0.55	0.29	0.27	3.94	0.69	6.31
	1 wk	15.80	2.83				0.63	8.64	0.84	8.20
	1 mo	17.40	2.91	9.37	1.68	1.45	1.64	7.31	0.58	5.04
14*	Baseline	3.77	1.61	0.52	0.39	11.80	0.69	1.94	0.06	6.41
	3 h	3.66	1.52	0.46	0.35	9.85	0.62	2.02	0.05	6.37
	1 wk	13.30	5.68	0.88	0.74	15.50	1.01	3.71	0.08	8.62
	1 mo	21.40	8.49	3.35	0.85	24.50	2.62	10.00	0.11	6.17
15*	Baseline	7.81	0.44	0.47	0.53	6.64	1.11	56.50	36.70	9.50
	3 h	7.15	0.40	0.35	0.57	5.04	1.09	50.20	34.40	8.07

(continued)

TABLE E3. (Continued)

Patient ID	Time point	slgE bee	slgE rApi m 1	slgE rApi m 2	slgE rApi m 3	slgE rApi m 5	slgE rApi m 10	slgE wasp	slgE rVes v 1	slgE rVes v 5
16*	1 wk	9.31	0.84	0.36	0.65	5.12	1.08	53.30	38.00	8.89
	1 mo	8.77	0.62	0.52	0.67	6.12	1.53	57.10	34.90	9.02
	Baseline	9.09	0.07	2.13	0.04	7.97	0.04	5.00	0.16	1.04
	3 h	8.48	0.07	2.30	0.07	6.63	0.04	4.92	0.14	0.94
17*	1 wk	15.40	0.69	4.15	0.18	9.09	0.04	5.99	0.16	0.92
	1 mo	27.10	0.48	12.90	0.25	12.30	0.09	8.62	0.19	0.83
	Baseline	1.37	0.09	0.00	0.16	0.90	0.36	17.80	3.43	13.30
	3 h	1.05	0.07	0.01	0.14	0.79	0.37	21.00	3.74	14.80
18	1 wk	18.00	1.97	0.01	0.97	3.47	9.45	47.90	1.26	59.20
	1 mo	15.40	1.50	0.01	0.57	3.03	7.96	46.80	0.79	55.90
	Baseline	5.78	0.02	0.03	0.51	6.78	0.06	26.60	10.50	8.84
	3 h	5.80	0.02	0.04	0.50	6.16	0.06	24.80	10.20	8.57
19*	1 wk	19.90	1.91	0.06	14.40	19.80	1.12	28.90	12.20	9.98
	1 mo	53.70	16.70	0.14	8.83	22.70	1.75	35.70	13.50	10.70
	Baseline	5.81	0.37	0.01	0.04	6.32	0.02	25.30	11.70	6.76
	3 h	5.86	0.38	0.02	0.06	5.73	0.02	26.50	12.20	7.10
20†	1 wk									
	1 mo									
	Baseline	2.73	0.10	0.55	0.21	0.13	0.20	15.10	0.11	13.10
	3 h	2.00	0.09	0.36	0.18	0.12	0.17	12.40	0.09	10.60
21*	1 wk	3.97	0.15	0.61	0.45	0.21	0.26	16.90	0.17	13.00
	1 mo	18.00	0.93	3.78	1.32	1.27	0.76	28.30	0.73	12.70
	Baseline	1.05	0.78	0.02	0.08	0.65	0.00	4.90	0.38	3.76
	3 h	0.90	0.66	0.03	0.08	0.57	0.01	4.62	0.36	3.40
22*	1 wk	1.94	0.78	0.09	0.21	1.72	0.04	4.84	0.31	3.07
	1 mo	3.40	0.57	0.18	0.16	2.30	0.06	4.97	0.30	2.72
	Baseline	13.80	2.08	0.05	1.99	7.78	6.78	37.30	0.61	44.50
	3 h	12.20	1.87	0.04	1.94	7.15	5.76	37.20	0.73	44.80
25*	1 wk	43.40	8.12	0.16	2.90	20.50	37.10	82.70	19.10	73.70
	1 mo									
	Baseline	0.73	0.06	1.26	0.00	1.04	0.01	13.80	0.10	13.50
	3 h	0.80	0.04				0.01	15.60	0.10	14.00
26	1 wk	1.45	0.52	1.94	0.05	1.11	0.01	15.80	0.10	13.50
	1 mo	3.63	0.62	9.39	0.02	1.22	0.02	13.30	0.07	11.40
	Baseline	0.63	0.04	0.13	0.06	0.54	0.26	1.26	0.07	1.50
	3 h	0.63	0.05	0.13	0.07	0.48	0.29	1.36	0.08	1.62
27*	1 wk									
	1 mo									
	Baseline	13.10	1.87	0.09	0.84	48.40	0.17	46.30	2.61	52.40
	3 h	15.20	1.94	0.04	0.73	36.80	0.18	46.50	2.79	57.30
28*	1 wk	27.50	9.34	0.15	2.00	63.80	1.02	43.70	4.51	53.50
	1 mo									
	Baseline	0.78	0.12	0.36	0.03	6.41	0.03	1.10	0.06	0.74
	3 h	0.64	0.11	0.27	0.04	4.93	0.03	0.93	0.06	0.72
29*	1 wk	7.96	2.75	3.61	0.32	9.84	0.49	1.74	0.08	0.74
	1 mo	7.21	1.92	3.11	0.22	11.60	0.34	1.76	0.12	0.72
	Baseline	3.77	0.46	0.25	0.09	2.93	0.05	8.81	2.80	3.75
	3 h	4.05	0.44	0.19	0.09	2.71	0.04	8.71	2.94	3.76
30	1 wk	17.80	13.40	2.48	0.19	5.04	0.13	12.60	5.82	5.49
	1 mo	8.67	5.82	1.40	0.13	3.20	0.12	9.67	4.22	3.63
	Baseline	7.25	0.01	0.32	0.02	2.35	0.01	152.00	162.00	29.40
	3 h	7.13	0.01	0.32	0.03	2.39	0.01	98.80	174.00	30.10
	1 wk	6.52	0.01	0.31	0.04	2.15	0.01	162.00	167.00	26.30
	1 mo	4.68	0.01	0.33	0.04	2.08	0.00	89.90	159.00	23.90

(continued)

TABLE E3. (Continued)

Patient ID	Time point	slgE bee	slgE rApi m 1	slgE rApi m 2	slgE rApi m 3	slgE rApi m 5	slgE rApi m 10	slgE wasp	slgE rVes v 1	slgE rVes v 5
31	Baseline	0.61	0.04	0.47	0.06	0.04	0.10	0.65	0.13	0.52
	3 h	0.75	0.03	0.54	0.07	0.06	0.11	0.67	0.13	0.49
	1 wk	0.71	0.05	0.59	0.08	0.07	0.13	0.66	0.21	0.53
	1 mo	0.38	0.03	0.35	0.05	0.04	0.07	0.36	0.11	0.26
32	Baseline	0.84	0.05	0.41	0.02	1.89	0.21	2.61	0.02	2.48
	3 h	0.83	0.05	0.41	0.03	1.66	0.20	2.60	0.02	2.41
	1 wk	1.06	0.10	0.53	0.04	1.92	0.24	2.34	0.04	2.36
	1 mo	1.33	0.22	0.95	0.03	1.49	0.46	1.86	0.02	1.64

*Patient with LLR after sting challenge.

†Patient with systemic reaction after sting challenge.