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Added sensitivity of component-resolved diagnosis in hymenoptera venom-allergic patients with elevated serum tryptase and/or mastocytosis

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Abstract

Background: Anaphylaxis caused by hymenoptera venom allergy is associated with elevation of baseline serum tryptase (sBT) and/or mastocytosis in about 5% of patients. Up to now, no information has become available on single venom allergen sIgE reactivity and the usefulness of component-resolved approaches to diagnose this high-risk patient group. To address the component-resolved sIgE sensitization pattern and diagnostic sensitivity in hymenoptera venom-allergic patients with elevated sBT levels and/or mastocytosis, a panel of yellow jacket and honeybee venom allergens was applied on a widely used IgE immunoassay platform.
Methods: Fifty-three patients with mastocytosis and/or elevated sBT tryptase level and systemic reactions to hymenoptera venoms were analyzed for their IgE reactivity to recombinant yellow jacket and honeybee venom allergens by Immulite3 g.
Results: sIgE reactivity to Ves v 1, Ves v 5, Api m 1 to Api m 4 and Api m 10 was found at a similar frequency in hymenoptera venom-allergic patients with and without elevated sBT levels and/or mastocytosis. However, the use of the recombinant allergens and a diagnostic cutoff of 0.1 kU_A/L allowed the diagnosis of patients with otherwise undetectable IgE to venom extract. The diagnostic sensitivity of yellow jacket venom allergy using the combination of Ves v 1 and Ves v 5 was 100%.
Conclusions: In high-risk patients with elevated sBT levels and/or mastocytosis, the use of molecular components and decreasing the threshold sIgE level to 0.1 kU_A/L may be needed to avoid otherwise undetectable IgE to hymenoptera venom extracts in about 8% of such patients.

Abbreviations
BM, bone marrow; CCD, cross-reactive carbohydrate determinant; CM, cutaneous mastocytosis; CRD, component-resolved diagnosis; HBV, honeybee venom; MIS, mastocytosis in the skin; sBT, baseline serum tryptase; sIgE, specific IgE; SM, systemic mastocytosis; YJV, yellow jacket venom.

Hymenoptera venom allergy is a potentially life-threatening disease mediated by the cross-linking of receptor-bound IgE antibodies on the surface of mast cells and basophils in allergic individuals. In recent large studies on patients with mastocytosis, a higher incidence of severe anaphylaxis following

hymenoptera stings than in the general population was documented (1–3).

Mastocytosis is a heterogeneous disorder characterized by proliferation and accumulation of mast cells in the skin, bone marrow (BM), and other tissues (4, 16). In recent years, an association of hymenoptera venom allergy, especially of severe allergic sting reactions, with mastocytosis was documented in 1–7.9% of patients with hymenoptera venom allergy and mastocytosis (1, 3, 5–10). The most frequent type of sensitization in patients with clonal mast cell disorders is to *Vespidae* (5). In addition, the level of baseline serum tryptase (sBT) in patients with hymenoptera venom is associated with more severe reactions following hymenoptera stings (1, 2).

Tryptase is a mast cell mediator, present in two major forms: alpha and beta. The beta tryptase is stored in mast cell granulae and released during mast cell activation (11). The baseline level of tryptase in serum is closely related to the total load of mast cells in the body (12).

There are several inherent problems in the management of patients with hymenoptera venom-allergic patients with elevated sBT levels and/or mastocytosis, both in diagnosis and in applying immunotherapy.

Patients with elevated sBT levels and/or mastocytosis have lower total IgE levels as compared to the general population (13, 14). When these patients have hymenoptera allergy, negative IgE and negative skin tests appear to be quite common, restricting them from otherwise indicated hymenoptera venom immunotherapy (VIT).

During hymenoptera venom immunotherapy, side-effects are more frequent in patients with mastocytosis, especially in those with yellow jacket venom (YJV) allergy, compared with the general hymenoptera venom-allergic population (15). According to different studies in which sting challenge and/or field sting reactions of yellow jacket venom-allergic patients who have underwent VIT were analyzed, the protection rate of VIT in patients with mastocytosis and/or elevated sBT level varies from 15 to 85% (1, 3, 7, 9, 17, 18), with an average protection rate of 72%. This is a much lower success rate compared to 95% for yellow jacket venom-allergic patients without mastocytosis (19). These findings indicate a lower efficacy of VIT, especially in yellow jacket venom-allergic patients with mastocytosis and/or elevated sBT compared to yellow jacket venom-allergic patients without this diagnosis.

So far, no information has been available on the sIgE reactivity pattern to hymenoptera venom allergen components in patients with elevated sBT levels and/or mastocytosis and history of systemic sting reactions. Because special reactivity pattern might be a potential explanation of the higher susceptibility to develop hymenoptera venom allergy and of reduced efficacy of VIT in mastocytosis patients, here we analyzed the sensitization profiles of those patients with a panel of cross-reactive carbohydrate determinant (CCD)-free yellow jacket and honeybee venom (HBV) allergens on an established sIgE immunoassay platform (20). Component resolution revealed no obvious differences in the reactivity profiles of hymenoptera venom-allergic patients with and without elevated sBT levels and/or mastocytosis. However, increased diagnostic sensitivity was observed when a threshold of 0.1 kU_A/l was used on an allergen-resolved level in patients with increased sBT or mastocytosis and undetectable or low sIgE to hymenoptera venom extract or unclear skin test results.

Methods

Patients

The study group contained 53 patients (26 male/27 female, age 18-76, median age 55) with allergy to hymenoptera venom and increased sBT level and/or mastocytosis, and the control group contained 26 hymenoptera venom-allergic patients (11 male/15 female, age 24–80, median age 57) without increased sBT level and/or mastocytosis.

Diagnosis of hymenoptera venom allergy was based on a combination of a clinical history of an anaphylactic sting reaction, a positive intradermal skin test, and/or positive sIgE levels to hymenoptera venom extracts (HBV, i1, and YJV, i3).

The diagnosis of mastocytosis was made according to WHO criteria (4). Serum tryptase was measured at least 2 weeks after a sting event using a commercial fluorimetric assay (Thermo Fisher Scientific, Uppsala, Sweden) and the threshold set at 11.4 ng/ml. Bone marrow biopsies were conducted in 24 patients and smears examined for the presence of atypical mast cells according to the guidelines (21). Bone marrow mast cells were analyzed for the expression of CD25 by immunofluorescence as described previously (4) and the activating c-kit mutation D816V detected by PCR (22). All patients had given informed written consent, and the study was approved by the local ethics committee.

Allergens

Api m 1, Api m 2, Api m 3, Api m 10, Ves v 1, and Ves v 5 were recombinantly produced as secreted full-length CCD-free proteins in *Spodoptera frugiperda* (Sf9) insect cells and purified by nickel-chelating affinity chromatography as previously described (23–27). Api m 4 was generated by peptide synthesis. All allergens were used for the generation of research prototype allergen immunoassays (Siemens Healthcare Diagnostics, Tarrytown, NY,USA).

Immunoreactivity of patient sera

sIgE reactivity was analyzed on an Immulite2000 platform (Siemens Healthcare Diagnostics) using commercially available assays for HBV (i1) and YJV (i3) (Siemens Healthcare Diagnostics) and clinical research prototype immunoassays for Api m 1, Api m 2, Api m 3, Api m 4, Api m 10, Ves v 1, and Ves v 5 (Siemens Healthcare Diagnostics).

Results

Clinical data of patients

The study group contained 53 patients with elevated sBT levels and/or mastocytosis and a history of hymenoptera

Table 1 Demographic and clinical data of the hymenoptera venom-allergic patients

	Mastocytosis/ sBT group	Normal tryptase group
Total	53	26
Sex		
Male	26	11
Female	27	15
Age		
Mean (SD)	54,4 (14.07)	53.9 (15.7)
Median (range)	55 (18–76)	57 (24–80)
Tryptase ng/ml, mean (SD)	31.9 (37.4)	5.4 (2.1)
Total IgE kU/l, median (range)	56.7 (2.3–8496)	89 (10–2551)
Mastocytosis disorder		
Systemic mastocytosis	17	0
Cutaneous mastocytosis	5	0
Mastocytosis in the skin	4	0
MMAS	1	0
Grade* of allergic reaction		
I	3	6
II	17	5
III	20	13
IV	9	2
unknown	4	0

*According to Ring and Messmer (40).

venom allergy. Of those, 49 had a history of YJV and four patients a history of HBV hypersensitivity. The demographic and clinical data of patients are summarized in Table 1. Fifty-one of the patients had sBT levels of higher than 11.4 ng/ml (Fig. 1). The other two were included into the study group due to a clear diagnosis of cutaneous mastocytosis (CM). Seventeen patients were diagnosed with systemic mastocytosis (SM), and one exhibited an activating c-KIT mutation with monoclonal mast cell activation syndrome (MMAS). Nine additional patients were diagnosed with CM or mastocytosis in the skin (MIS). BM biopsy was performed in 24 patients. The clinical characteristics of the patients of the study group are shown in detail in Table 2.

Diagnostic sensitivity of sIgE to YJV allergens Ves v 1 and Ves v 5

The study group and the control group contained 49 and 25 patients, respectively, for whom yellow jackets were clearly identified as the culprit insect eliciting a systemic allergic reaction. For these patients, the diagnostic sensitivity of YJV extract and of the allergens Ves v 1 and Ves v 5 was addressed (Fig. 2). Using YJV extract, the diagnostic sensitivity in the study group was 91.8% using a cutoff of 0.1 kU_A/l and 87.8% using a cutoff of 0.35 kU_A/l, respectively. Four patients showed sIgE levels below 0.1 kU_A/l. In contrast, in the control group, all patients could be diagnosed using YJV extract and a cutoff of 0.1 kU_A/l (92% with the cutoff of 0.35 kU_A/l).

When using the cutoff of 0.35 kU_A/l, the diagnostic sensitivity of the allergens was unexpectedly low in the study group (63.3% for Ves v 1 and 85.7% for Ves v 5). Decreasing the cutoff to 0.1 kU_A/l, sIgE reactivity with the allergens Ves v 1 and Ves v 5 was found in the study group at a prevalence of 81.6% and 98%, respectively. In stark contrast, in the control group, the diagnostic sensitivity of Ves v 1 and Ves v 5 was 72% and 92%, respectively, regardless of which cutoff was used. Interestingly, in the group of patients with elevated sBT levels and/or mastocytosis, there is a relevant portion of patients exhibiting sIgE levels against the allergens in the range between 0.1 and 0.35 kU_A/l compared to none in the control group. However, by using the combination of the two major YJV allergens Ves v 1 and Ves v 5 and a cutoff of 0.1 kU_A/l, the diagnostic sensitivity could be raised to 100% in the study group and the control group, respectively. Using the traditional cutoff of 0.35 kU_A/L, four patients in the study group (8.2%) with severe systemic reactions would have been completely negative in *in vitro* sIgE measurement, while none in the control group would have been missed.

IgE reactivity to YJV and HBV allergens in patients with and without elevated sBT levels and/or mastocytosis

To examine whether patients of the study group and the control group differ in their IgE reactivity profile to individual allergens, the patients were divided into different groups based on a combination of clinical history, skin test, and sIgE to HBV and YJV extract. Although most of the patients had a systemic reaction after a yellow jacket sting, patients with a double-positive skin test and/or detectable specific IgE to YJV and HBV were classified as double-positive. For sIgE measurements, a cutoff of 0.1 kU_A/l was used, which has previously been established as a suitable lower-end cutoff on the Immulite2000 immunoassay platform (20). The study group contained 29 patients, who were sensitized to YJV only, whereby three patients without detectable sIgE were included due to an anaphylactic reaction of grade II or III after sting by an YJ. Twenty patients were double-positive to YJV and HBV, and only four patients were monosensitized to HBV. The control group consisted of nine patients monosensitized to YJV and 17 with double-positive test results.

All patient populations exhibited comparable reactivity with the YJV major allergens Ves v 1 and Ves v 5. Using the cutoff of 0.1 kU_A/l, 82.8% of the YJV-monosensitized and 80% of the double-positive patients of the study group showed IgE reactivity with Ves v 1 (Fig. 3A,B), which was comparable with 77.8 and 70.6% in the control group (Fig. 3D,E). IgE reactivity to Ves v 5 was detected in 96.6 and 100% of the patients of the study group (Fig. 3A,B) and in 88.9 and 94.1% of patients of the control group (Fig. 3D, E). The IgE reactivity of the different patient groups with the YJV allergens using the cutoffs of 0.1 and of 0.35 kU_A/l is summarized in Fig. 3F. The detailed reactivity profiles of the patients are shown in Table S1.

Table 2 Characteristics of hymenoptera venom-allergic patients with mastocytosis and/or elevated baseline serum tryptase

Pt ID	Age	Sex	Grade of sting reaction*	Serum tryptase (ng/ml)	BM biopsy	BM mast cells CD25+	Activating KIT mutation	Spindle-shaped mast cells	Skin morphology	Mast cell disorder
1	50	M	II	13.7	Neg	Neg	Neg	Neg	Neg	
2	59	M	IV	14.8	n.d.	n.d.	n.d.	n.d.	Neg	
3	26	W	III	22.2	n.d.	n.d.	n.d.	n.d.	Pos	MIS
4†	59	W	II	20.4	n.d.	n.d.	n.d.	n.d.	Neg	SM
5	56	W	I	17.9	Pos	Neg	n.d.	Pos	Neg	SM
6	73	W	II	21.0	n.d.	n.d.	n.d.	n.d.	Neg	
7	31	W	II	18.6	Neg	Neg	Neg	Neg	Pos	CM
8	55	M	III	28.7	n.d.	n.d.	n.d.	n.d.	Neg	
9	44	M	III	73.8	Pos	n.d.	n.d.	Neg	Pos	SM
10	39	W	n.d.	4.8	Neg	Neg	Neg	Neg	Pos	CM
11	63	W	II	13.5	n.d.	n.d.	n.d.	n.d.	Neg	
12	60	M	IV	27.4	Pos	n.d.	n.d.	Neg	Pos	SM
13	46	W	III	42.4	Pos	n.d.	n.d.	Neg	Neg	SM
14	62	W	I	61.3	Pos	n.d.	n.d.	Neg	Pos	SM
15	72	W	n.d.	14.3	n.d.	n.d.	n.d.	n.d.	Neg	
16	76	W	III	11.6	n.d.	n.d.	n.d.	n.d.	Neg	
17	44	M	III	13.0	n.d.	n.d.	n.d.	n.d.	Neg	
18	66	W	III	18.9	Pos	n.d.	n.d.	Pos	Pos	SM
19	59	W	III	177.0	Pos	Neg	Pos	Pos	Neg	SM
20	42	W	II	11.7	n.d.	n.d.	n.d.	n.d.	Neg	
21	41	M	n.d.	52.1	Pos	Pos	Pos	Neg	Pos	SM
22	42	M	III	24.2	n.d.	n.d.	n.d.	n.d.	Pos	MIS
23	55	W	I	11.7	n.d.	n.d.	n.d.	n.d.	Neg	
24	69	W	II	13.1	n.d.	n.d.	n.d.	n.d.	Neg	
25	55	W	III	11.7	Pos	Pos	Pos	Pos	Neg	SM
26	56	M	II	50.4	Pos	Pos	Neg	Pos	Neg	SM
27	66	W	III	149.0	Pos	Neg	Neg	Neg	Pos	SM
28	49	M	III	81.0	n.d.	n.d.	n.d.	n.d.	Pos	MIS
29	66	W	II	11.6	n.d.	n.d.	n.d.	n.d.	Neg	
30	65	W	III	14.0	Neg	Neg	Neg	Neg	Neg	
31†	47	M	IV	13.2	n.d.	n.d.	n.d.	n.d.	Neg	SM
32	44	M	II	7.3	Neg	Neg	Neg	Neg	Pos	CM
33	64	M	III	23.3	n.d.	n.d.	n.d.	n.d.	Neg	
34	68	M	III	11.8	n.d.	n.d.	n.d.	n.d.	Neg	
35	19	M	III	13.6	n.d.	n.d.	n.d.	n.d.	Neg	
36	73	W	II	25.9	n.d.	n.d.	n.d.	n.d.	Neg	
37	53	M	IV	29.7	Pos	Pos	Pos	Neg	Neg	SM
38	46	M	III	14.9	n.d.	n.d.	n.d.	n.d.	Neg	
39	68	W	II	17.4	n.d.	n.d.	n.d.	n.d.	Neg	
40†	46	M	IV	128.0	n.d.	n.d.	n.d.	n.d.	Pos	SM
41	75	M	III	27.5	Neg	Neg	Pos	Neg	Neg	MMAS
42	49	M	IV	12.6	Neg	Neg	Neg	Pos	Neg	
43	70	M	IV	12.7	n.d.	n.d.	n.d.	n.d.	Neg	
44	69	W	III	12.6	n.d.	n.d.	n.d.	n.d.	Neg	
45	55	W	II	16.1	Pos	Pos	Pos	Pos	Neg	SM
46	66	M	IV	11.5	n.d.	n.d.	n.d.	n.d.	Neg	
47	54	M	III	14.9	n.d.	n.d.	n.d.	n.d.	Neg	
48	41	M	II	13.8	Neg	Neg	Neg	Neg	Neg	
49	46	W	II	18.9	Pos	Neg	Neg	Neg	Pos	CM
50	76	W	n.d.	25.0	Pos	Neg	n.d.	Pos	Pos	SM
51	18	M	II	17.2	n.d.	n.d.	n.d.	n.d.	Neg	
52	36	M	IV	38.8	n.d.	n.d.	n.d.	n.d.	Pos	MIS
53	56	W	II	140.0	Pos	n.d.	n.d.	Neg	Pos	CM

BM, bone marrow; CM, cutaneous mastocytosis; MIS, mastocytosis in the skin; MMAS, monoclonal mast cell activation syndrome; n.d., not determined; SM, systemic mastocytosis. Patients 1–29 are monosensitized to YJV, patients 30–49 are sensitized to YJV and HBV, and patients 50–53 are monosensitized to HBV.

*According to Ring and Messmer (40).

†The patient came to clinic with an existing diagnosis of mastocytosis.

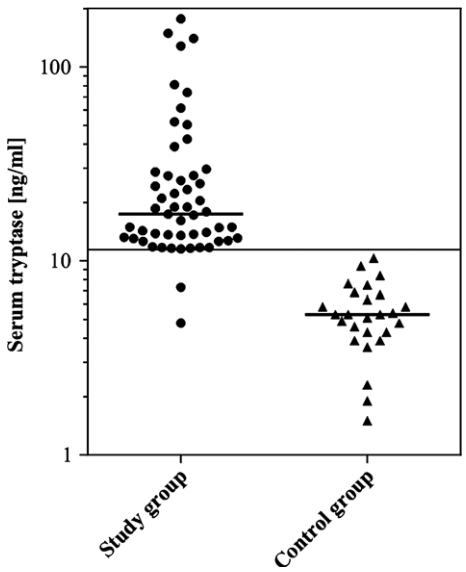

Figure 1 Serum tryptase levels of hymenoptera venom-allergic patients. Serum tryptase levels of the study group (n = 53) and the control group (n = 26) as measured at least 2 weeks after the last episode of a sting reaction. The mean of the study group is 31.93 ± 37.43 ng/ml and of the control group 5.43 ± 2.12 ng/ml. The assay threshold value set at 11.4 ng/ml is represented by a solid line.

Moreover, the IgE reactivity with the HBV allergens Api m 2, Api m 3, Api m 4, and Api m 10 (Fig. 3B,E) was comparable to that described in a former study for patients with a primary sensitization to HBV (28). Except for the HBV major allergen Api m 1, the reactivity was lower compared to other studies most likely reflecting the different patient selection (28–30).

Reactivity profiles of patients with low or undetectable sIgE to hymenoptera venom extract

Among the patients of the study group, four patients (Fig. 4A, patients 12, 27, 28, and 29) with grade II to IV systemic reactions to yellow jacket stings had sIgE levels to YJV below 0.1 kU_A/L. Three of these patients were diagnosed with a mastocytosis disorder, and two additionally exhibited negative intracutaneous skin tests with YJV. All these patients showed sIgE to Ves v 5 (one additionally to Ves v 1) in the range between 0.1 and 0.35 kU_A/l. Two patients with systemic mastocytosis exhibited YJV-specific IgE in the range between 0.1 and 0.35 kU_A/L (Fig. 4A, patients 21 and 25). One of them showed sIgE reactivity to Ves v 1 above 0.35 kU_A/l and one with Ves v 5, respectively. Additionally, one patient of the control group with sIgE to YJV below 0.35 kU_A/l could be clearly diagnosed using Ves v 5 and another one by using Ves v 5 and Ves v 1 (Fig 4A, patients 55 and 62).

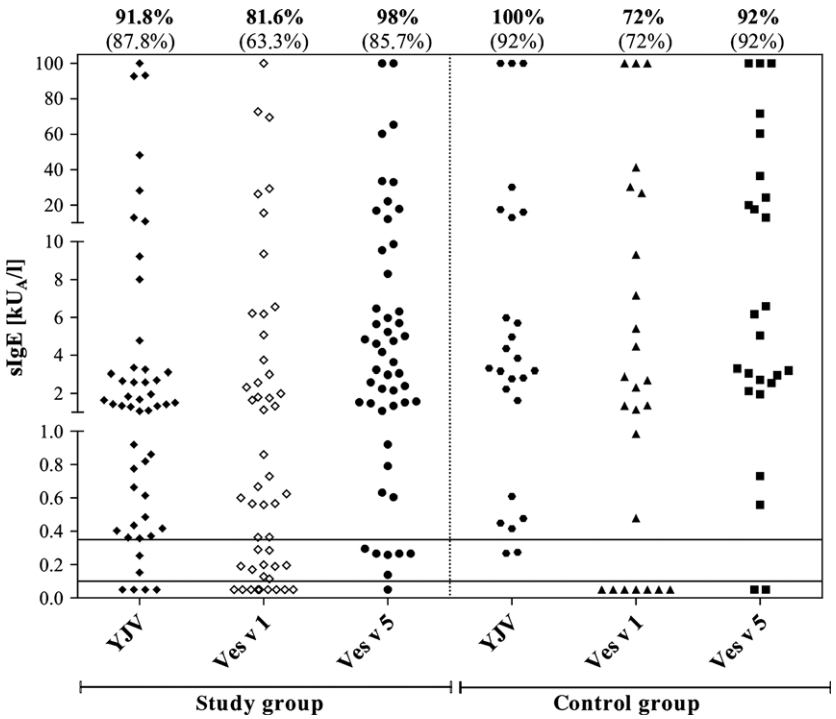

Figure 2 sIgE reactivity of individual sera using extract or recombinant allergens from patients with systemic reactions after YJ stings. IgE reactivity to YJV extract or recombinant YJV allergens (Ves v 1, Ves v 5) of sera from YJV-allergic patients with (study group) and without (control group) elevated sBT level and/or mastocytosis. The lower-end cutoffs of 0.1 kU_A/l and 0.35 kU_A/l are presented as solid lines. Percentages in boldface and in parentheses indicate the IgE reactivity of allergens using the cutoff of 0.1 and 0.35 kU_A/l, respectively.

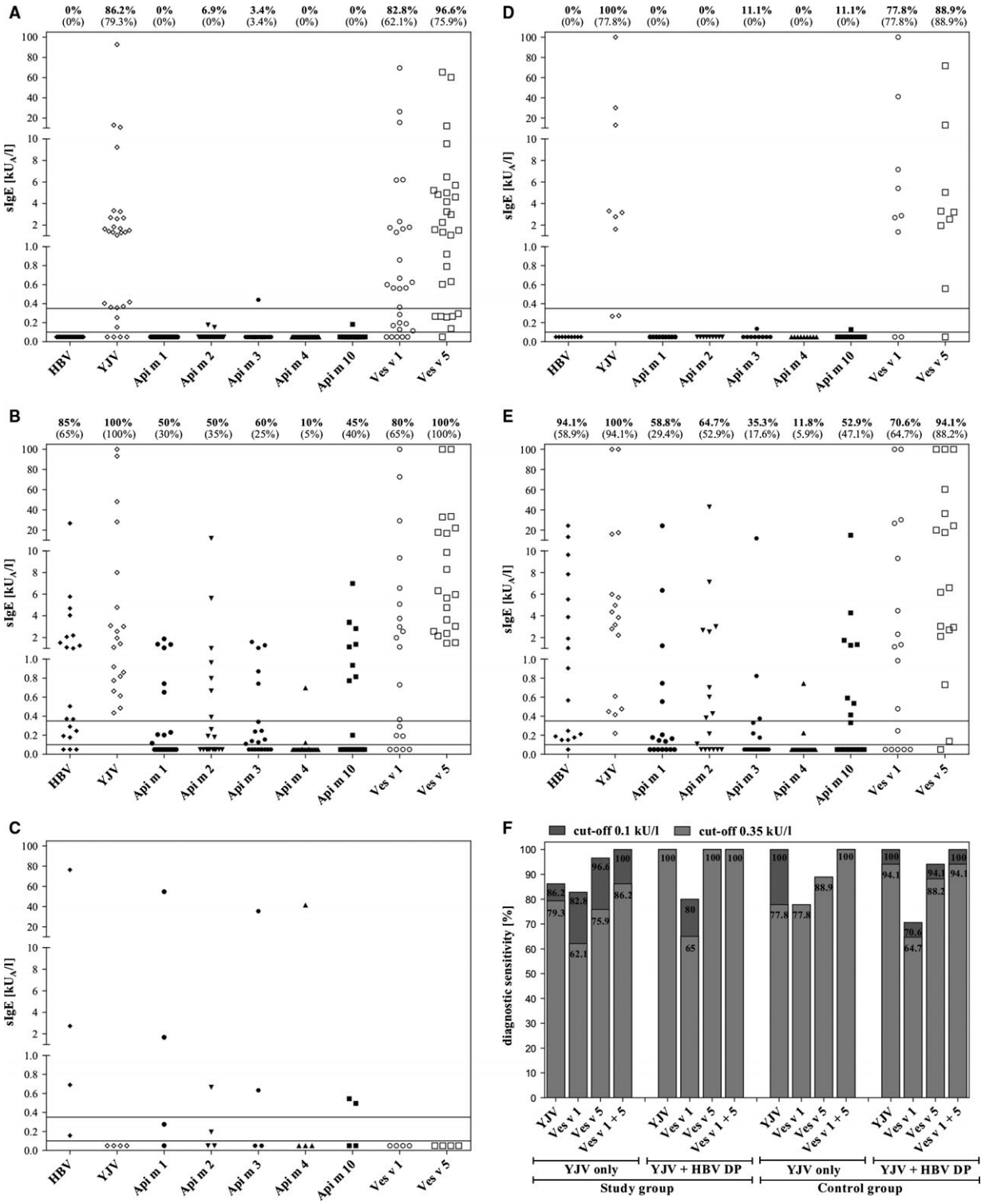

Figure 3 Immunoreactivity of patient sera with individual allergens and venom extracts. IgE reactivity to HBV and YJV allergens and venom extracts of sera from hymenoptera venom-allergic patients with (A–C) and without (D,E) elevated sBT level and/or mastocytosis. (A,D) Patients monosensitized to YJV. (B,E) patients sensitized to YJV and HBV. (C) Patients monosensitized to HBV. The lower-end cutoffs of 0.1 and 0.35 kU_A/l are presented as solid lines. Percentages in boldface and in parentheses indicate the IgE reactivity of allergens using the cutoff of 0.1 and 0.35 kU_A/L, respectively. (F) Diagnostic sensitivity of sIgE to YJV, Ves v 1, and Ves v 5 using the different cutoffs.

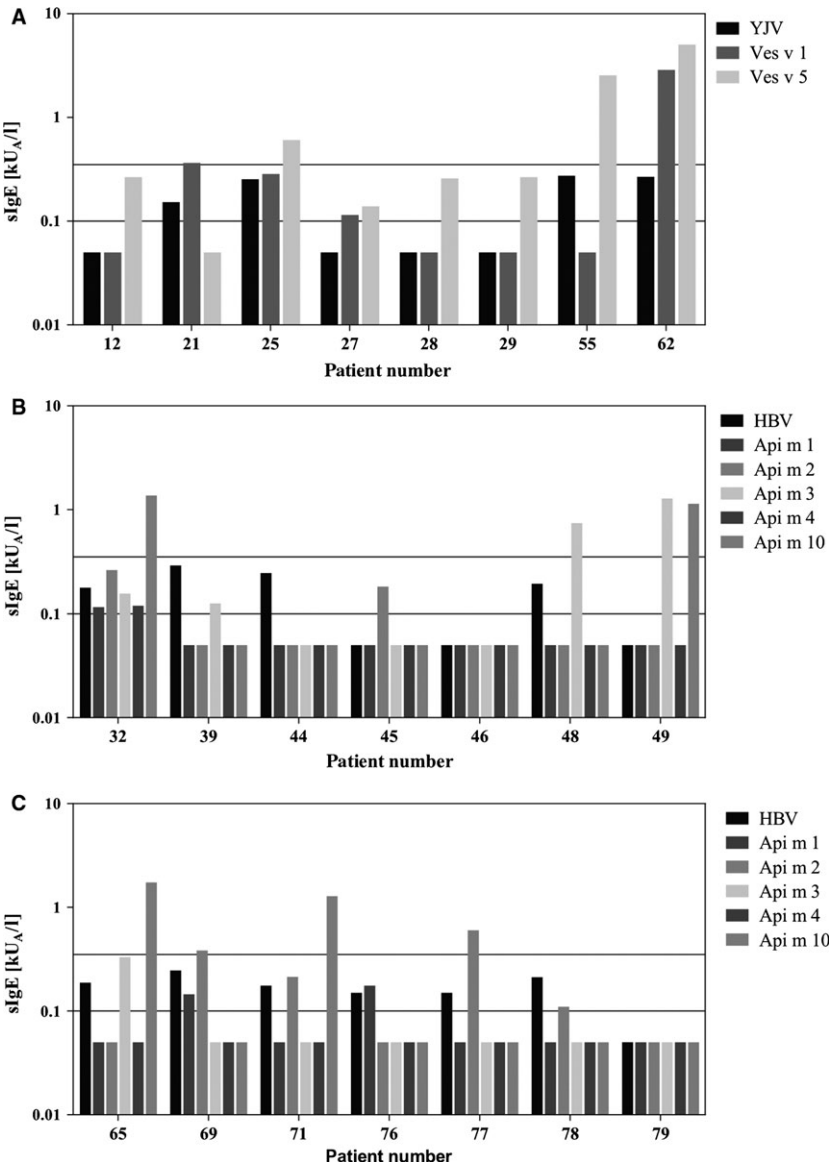

Figure 4 sIgE reactivity using recombinant allergens of individual patients with low or undetectable sIgE to hymenoptera venom extract. (A) Reactivity with YJV allergens (Ves v 1, Ves v 5) of patients with clear-cut clinical history of YJV allergy. (B,C) Reactivity with HBV allergens (Api m 1, Api m 2, Api m 3, Api m 4, Api m 10) of patients with clinical history of YJV allergy and additional evidence for a sensitization to HBV. (B) Patients of the study group with elevated sBT level and/or mastocytosis. (C) Patients of the control group without elevated sBT level and/or mastocytosis. The lower-end cutoffs of 0.1 and 0.35 kU_A/L are presented as solid lines.

We additionally analyzed the reactivity profile with HBV allergens of patients of the study group (Fig. 4B) and control group (Fig. 4C) double-positive for YJV and HBV and low or undetectable sIgE to HBV. One patient (patient 49) who had HBV-specific IgE of <0.1 kU_A/l (but in a former measurement 0.5 kU_A/l) exhibited a significant reactivity with Api m 3 and Api m 10. Five additional patients (patients 32, 39, 48, 65, and 71) with sIgE to HBV between 0.1 and 0.35 kU_A/l also exhibited reactivity with Api m 3 and/or Api m 10 with values above 0.35 kU_A/L (except patient 39). Interestingly, patient 71 in 2010 had sIgE to HBV of 0.93 kU_A/L and patient 48 in 2008 and 2010 of 5.64 and 0.51 kU_A/l, respectively, hinting to a history of HBV allergy. Three patients (45, 77, and 78) reacted with Api m 2 only, which might be explained by cross-reactivity with Ves v 2, the homologue from YJV. One patient (44) with low sIgE to HBV and two (46 and 79) with a positive skin test to HBV showed no reactivity with any of the HBV allergens, and only two patients (69 and 76) exhibited a slight reactivity with Api m 1. Patient 69 additionally showed reactivity with Api m 2 above 0.35 kU_A/L and, in addition to a history of YJV allergy, showed a mild reaction (grade I) after a honey-

bee sting, indicating that low-level sIgE reactivity might also be of clinical relevance.

Discussion

In this study, we addressed, for the first time, the component resolution of sIgE reactivity to a broad panel of recombinant YJV and HBV allergens of hymenoptera venom-allergic patients with elevated sBT levels and/or mastocytosis. Hymenoptera venom allergy represents the most common trigger for anaphylaxis in patients with mastocytosis (6), and moreover, mastocytosis patients and those with increased sBT levels are at risk for more severe sting reactions (10, 32, 33). The frequency of mastocytosis in patients with hymenoptera venom allergy is in the range between 1 and 7.9% (1, 3, 5, 7), which is substantially higher than in the general population with a range between 0.00125 and 0.07% (7). Five to 19% of patients with mastocytosis suffer from hymenoptera venom allergy (6, 34).

The only causative treatment that is effective in reducing the risk of subsequent systemic reactions in hymenoptera venom-allergic patients is venom immunotherapy (VIT). A prerequisite for VIT is the demonstration of a sensitization by sIgE or skin test. However, proper diagnosis of hymenoptera venom allergy in mastocytosis patients is in some cases problematic because total IgE levels are lower (13) and sIgE and skin tests might be more often negative compared to hymenoptera venom-allergic patients without mastocytosis.

To date, there is only scarce knowledge about the pathogenic mechanisms underlying the association between mastocytosis and hymenoptera venom allergy. In addition, there is a well-documented reduced therapeutic efficacy of VIT in mastocytosis patients (19). One potential hypothesis is that specific sIgE sensitization patterns in addition to special characteristics of the disease account for these phenotypes of higher susceptibility to develop hymenoptera venom allergy and of reduced efficacy of VIT in mastocytosis patients. To date, no data about the sIgE reactivity profiles with particular allergenic components have become available for patients with elevated sBT levels and/or mastocytosis.

Hence, in this study, we examined hymenoptera venom-allergic patients with elevated sBT levels and/or mastocytosis for their sIgE reactivity profiles with recombinant YJV (Ves v 1 and Ves v 5) and HBV (Api m 1-4 and Api m 10) allergens. For the analysis of the particular sIgE reactivity, we used venom allergens that were recombinantly produced in *Spodoptera frugiperda* (Sf9) insect cells and that therefore allow the detection of allergen-specific IgE without the interference of cross-reactive carbohydrate determinants (23, 24, 26, 35), which represent a major concern for the specificity of diagnostic tests in hymenoptera venom allergy (36–38). The recombinant allergens were used for the generation of clinical research prototype immunoassays and analyzed on random-access automated immunoassay platform capable of measuring sIgE (Immulite2000 system, Siemens Healthcare Diagnostics) (20).

The analyses of sIgE reactivity on a component-resolved level revealed no obvious differences in the reactivity profiles of hymenoptera venom-allergic patients with and without elevated sBT levels and/or mastocytosis. This was true not only for the reactivity with the YJV major allergens Ves v 1 and Ves v 5 but also for the reactivity with the HBV allergens Api m 1-4 and Api m 10, thus pointing to the conclusion that the immunologic specificity does not account for the observed differential phenotypic aspects of disease risk, severity, and VIT outcome.

Because most of the patients included in this study had a clinical history of a systemic reaction to YJV (only few to HBV), their reactivity to YJV allergens was of special interest. Interestingly, we found that in stark contrast to the control group, a large portion of patients with elevated sBT levels and/or mastocytosis had sIgE levels against the allergenic components in the range between 0.1 and 0.35 kU_A/l. Only by applying a diagnostic cutoff of 0.1 kU_A/l and a combination of the major allergens Ves v 1 and Ves v 5, we were able to reach a diagnostic sensitivity of 100% in the both patient groups. In contrast, using the cutoff of 0.35 kU_A/l, four patients of the elevated sBT levels and/or mastocytosis group (8.2%, none in the control group) would have been completely negative in sIgE diagnostics and two of these patients also with negative intradermal skin test would not meet the inclusion criteria for VIT, despite having a history of a severe systemic reactions after stings by YJ. This demonstrates the added value of component-resolved diagnosis for patients with undetectable sIgE to venom extract as already shown previously (39). Such an added value of increased sIgE assay sensitivity might be especially important for patients with elevated sBT levels and/or mastocytosis. It is a common finding in those patients that hymenoptera venom extract-specific IgE is negative, which has been primarily attributed to an increased adsorption of IgE to the high-affinity IgE receptors on the surface of the large number of mast cells (1, 14). In addition, we also observed an incomplete representation of Ves v 5-specific IgE by the YJV extract as compared to the recombinant allergen component Ves v 5, a finding that has been described previously by others for the measurements of sIgE in YJV-allergic patients on the ImmunoCAP system (31). Moreover, in two patients with a sIgE level to YJV extract of <0.35 kU_A/l, we observed a similar finding for Ves v 1-specific IgE (patients 21 and 62).

Admittedly, it is a matter of debate which diagnostic cutoff is reasonable for the detection of relevant sIgE sensitization. Our data, however, indicate that it might be advantageous to use the cutoff of 0.1 kU_A/l in patients with low amounts of circulating sIgE due to an overload of mast cells in the body and who are at a particular high risk to suffer from severe or even fatal anaphylactic reaction to another insect sting. It has been demonstrated previously that sIgE concentrations above the 0.1 kU_A/l lower-end threshold value can be measured reproducibly on major sIgE immunoassay platforms such as the ImmunoCAP system or the Immulite2000 system, which was used in this study (20).

In this study, we were also able to detect a significant sIgE reactivity to HBV allergens in patients with low or undetectable sIgE to HBV extract, especially to the allergens Api m 3 and Api m 10, which were previously shown to be

underrepresented in several therapeutic venom preparations (24) and induce lower levels of sIgG4 under VIT with honey bee venom extract (28). These therapeutic venom preparations are also commonly used for skin testing, a fact that even might explain the negative skin test with HBV of most of the patients who are sensitized to those allergens exclusively. However, due to the fact that the vast majority of the study patients were only stung by yellow jackets, the clinical relevance of these sensitizations remains unclear.

In summary, our data demonstrate that although no obvious differences can be found in the sIgE reactivity profile itself with routine or research prototype hymenoptera venom allergens available so far when comparing hymenoptera venom-allergic patients with or without elevated sBT levels and/or mastocytosis, there is a diagnostic advantage and added value of recombinant allergens in combination with a lower-end assay cutoff of 0.1 kU_A/l for the diagnosis of patients with low or undetectable sIgE to venom extract or unclear skin test results, especially for patients with elevated sBT levels and/or mastocytosis.

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Author contributions

JM performed the experiments and analyzed the data; KB coordinated the recruitment of mastocytosis patients, collected and analyzed the data, and provided critical revision of the manuscript; UD coordinated the recruitment of hymenoptera venom-allergic patients, collected and analyzed the data, and provided critical revision of the manuscript; JR coordinated the recruitment of patients and revised the final version of the manuscript; CS-W contributed to the interpretation of data and revised the final version of the manuscript; TG produced recombinant allergens and provided critical revision of the manuscript; SB supervised the study, analyzed the data, designed the figures, and wrote the manuscript; MO initiated and supervised the study, analyzed the data, and wrote the manuscript.

Conflicts of interest

KB has received payments from Thermo Fisher for oral presentations at company seminars and for controlling of company-initiated webinars for integrity. TG is an employee of PLS-Design GmbH. MO reports personal fees from Thermo Fisher, from Siemens Healthcare Diagnostics, and from Hitachi Chemical Diagnostics, and is co-founder of PLS-Design GmbH. The other authors declare that they have no conflict of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Serological data of patients assessed in sIgE reactivity analysis.

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