

Basal serum tryptase as risk assessment for severe Hymenoptera sting reactions in elderly

E. Guenova^{1,2}, T. Volz¹, M. Eichner³, W. Hoetzenecker¹, U. Caroli¹, G. Griesinger¹, G. Burow⁴, V. Mitev² & T. Biedermann¹

¹Department of Dermatology, Eberhard Karls University, Tübingen, Germany; ²Department of Biochemistry, Medical University, Sofia, Bulgaria; ³Department of Medical Biometry, Eberhard Karls University, Tübingen, Germany; ⁴Phadia GmbH, Freiburg, Germany

To cite this article: Guenova E, Volz T, Eichner M, Hoetzenecker W, Caroli U, Griesinger G, Burow G, Mitev V, Biedermann T. Basal serum tryptase as risk assessment for severe Hymenoptera sting reactions in elderly. *Allergy* 2010; DOI: 10.1111/j.1398-9995.2009.02302.x.

Keywords

age; anaphylactic reaction; hymenoptera venom; severity; tryptase.

Correspondence

T. Biedermann
Department of Dermatology, Eberhard Karls University, Tübingen, Germany.
Tel.: +49 7071 2980836
Fax: +49 7071 295708
E-mail: Tilo.Biedermann@med.uni-tuebingen.de

Accepted for publication 16 November 2009

DOI: 10.1111/j.1398-9995.2009.02302.x

Edited by: Hans-Uwe Simon

Abstract

Background: Epidemiologic studies suggest that elderly people are more prone to develop severe anaphylactic reactions. However, the exact cause for this phenomenon remains unclear.

Aims of the study: To study the role of the serum tryptase as a diagnostic parameter for individual risk evaluation and its impact on the severity of allergic reactions in elderly people.

Methods: Two hundred and seventy-four consecutive patients visiting the Department of Dermatology, Tübingen, Germany, who were diagnosed with honeybee or wasp venom allergy, were included in the study.

Results: Sting reaction severity increased with increased age and tryptase levels ($P = 0.001$ and $P = 0.0003$, respectively). Furthermore, we find not only a general increment in tryptase levels in elderly people ($P = 0.0001$) but also a continuous increase in tryptase concentrations even below the cut-off (11.4 µg/l) with increasing age ($P = 0.0026$).

Conclusions: Our data confirm serum tryptase as a risk factor for severe anaphylactic reaction to hymenoptera stings. Furthermore, we give first evidence that basal serum tryptase levels increase continuously with age and being an indicator for either increased mast cell load or reactivity this can at least partly be responsible for the observed aggravated allergic reactions in elderly people. As those patients are at increased risk for life-threatening anaphylactic reactions, it should be considered to adjust VIT especially in elderly patients with elevated tryptase levels as recommended for patients with mastocytosis by increasing venom doses during VIT and by considering its life-long continuation.

Up to 5% of the adult population in the Western countries develop anaphylactic reaction to honeybee or wasp stings and reactions to stings may vary broadly from skin involvement to shock (1–3). The causes for these different reaction patterns are not completely understood. One explanation seems to be that severe anaphylactic hymenoptera sting reactions are often associated with mastocytosis, for which one diagnostic marker is elevated tryptase levels (4–8).

The protease tryptase, is expressed almost exclusively in mast cells and presents in several isoforms. Tryptase levels are a useful marker for estimating mast cell numbers and/or their activation status depending on the analyzed isoform (9). Whereas tryptase α is enzymatically inactive and

released continuously, tryptase β is secreted during mast cell degranulation (10). Tryptase γ (serine protease 31), tryptase δ (mast cell protease 7-like tryptase-3), and the brain-specific serine protease 4 (ϵ -tryptase) play no role as diagnostic measures.

Interestingly, recent studies have demonstrated that tryptase levels seem to function as an individual risk factor for increased allergic reactions even in patients without mastocytosis. Haeberli et al. (4) demonstrated increased tryptase in 7.3% of venom-allergic patients. Similar results were reported by Kucharewicz et al., (11) where increased basal serum tryptase levels were found in 11% of patients allergic to wasp or honey bee venoms. Furthermore, both studies evidenced a

significant correlation of basal serum tryptase levels with sting reaction severity (4, 11).

It is known that older people are at increased risk to develop severe anaphylactic reactions after stings (12–14). The reason behind this phenomenon remains unclear. It has been speculated that elderly people suffer from a coexisting cardiovascular disease, which in the end could contribute to life-threatening anaphylaxis (3, 6). Interestingly, Kucharewicz et al. (11) found elevated serum tryptase levels with increasing age. However, the clinical significance of this finding has never been investigated closely.

In this study, we investigate the role of the basal serum tryptase as diagnostic parameter for individual risk evaluation and its impact on the severity of allergic reactions in elderly people.

Materials and methods

Patients

Two hundred and seventy-four patients allergic to honeybee or wasp venom were included in the study at the Allergy unit of the Department of Dermatology, University of Tübingen, Germany. All patients, had a history of systemic allergic reactions to hymenoptera stings, had positive intracutaneous skin test and/or specific serum IgE antibodies to the respective venom. Based on clinical symptoms, the reactions were graded into four classes according to Mueller (grades of severity I–IV) (15).

Skin tests

All patients underwent cutaneous skin testings using ten-fold dilutions of wasp and honey bee extracts (ALK-Scherax, Hamburg, Germany) with concentrations ranging from 10^{-6} to 10^{-3} g/l. Results were considered positive with a wheal of 5 mm or more after 20 min at a concentration of 10^{-3} g/l or less.

Basal serum tryptase and venom-specific IgE antibodies

Blood samples were collected between 8 and 12 am, at least 4 weeks after the hymenoptera sting. Sera were aliquoted and frozen at -80°C until testing. Levels of mast cell-specific tryptase (α - and β -tryptase) and of venom-specific IgE were measured by UniCAP (System ImmunoCAP Tryptase[®] and UniCAP 100; Phadia AB, Uppsala, Sweden). An IgE value of >0.35 kU/l was regarded as positive. The detection limit for tryptase measurement was <1.0 $\mu\text{g/l}$. Levels of 11.4 $\mu\text{g/l}$ and more were considered to be elevated.

Statistics

The data were analyzed using JMP IN 5.1 (SAS Inc., Cary, NC, USA) statistical software. Univariate logistic regression analysis was performed to study the impact of serum tryptase levels or age as independent risk factors upon severity of sting reactions. Decadic logarithm ($\log_{10}$) of the serum tryptase levels was used throughout, because of the positively

skewed distribution of the data. Simple linear regression allowed us to investigate the relationship between age and tryptase levels. The differences between gender and serum tryptase levels were analyzed by means of two-sample *t*-test.

Results

Demographic and clinical characteristics of the patient collective

Two hundred and seventy-four consecutive patients, who presented to the Allergy Unit at the Department of Dermatology, University of Tübingen, Germany between 2004 and 2006, were included in this study. The group contained 153 men and 121 women diagnosed with honeybee or wasp venom allergy according to the classification of Müller.

The median age was 44.8 ± 16.8 . Fifty-four patients (29 m; 25 f) showed grade I reaction, 106 patients (57 m; 49 f) were classified as grade II, 66 (41 m; 25 f) as grade III, and 48 patients (26 m; 22 f) reported on grade IV reactions.

Demographic and clinical characteristics of the patients with elevated basal serum tryptase levels

Tryptase levels were elevated in a total of 10.9% (30) of all 274 patients. Cutaneous or systemic mastocytosis could be diagnosed in just 7.5% (4) of the patients with elevated serum tryptase levels (>11.4 $\mu\text{g/l}$) according to the WHO criteria published in 2002 and revised in 2008 (16, 17). Furthermore, detailed medical history (abdominal discomfort, nausea, vomiting, and diarrhea) allowed a working diagnosis of systemic mastocytosis in another 7.5% (4) patients with elevated serum tryptase levels but in these patients the diagnosis could not be proven according to the WHO criteria (Table 1).

Basal serum tryptase level in the risk assessment for severe anaphylactic sting reactions

Sting reaction severity increased significantly with increasing tryptase levels [univariate logistic regression yielded $P = 0.0003$ for odds ratio (OR) level 1 vs level 4]. The geometric mean of serum tryptase in patients with grade I allergic reactions was 4.27 $\mu\text{g/l}$, whereas the geometric mean of serum tryptase in patients who had suffered from grade IV reactions was 7.18 $\mu\text{g/l}$. Furthermore, tryptase concentrations above the cut-off level of 11.4 $\mu\text{g/l}$ were detected by just 7% of the patients with grade I allergic reaction. This percentage reached 21.4% in patients with grade IV allergic reaction (Fig. 1A).

Impact of age on the risk for severe anaphylactic sting reactions

In this study group, the risk of severe anaphylactic reactions increased significantly with rising serum tryptase levels and with increasing age as well [univariate logistic regression with $P = 0.001$ for odds ratio (OR) level 1 vs level 4]. On the mean, patients with grade I allergic reactions were 38.9 years old, whereas patients with grade IV reaction were 51.5 years

Table 1 Demographic and clinical characteristics of the patients with elevated serum tryptase levels

ID	Gender	Age	Medical history	Serum tryptase ($\mu\text{g/l}$)	Severity of sting reaction (Grade I–IV)
1	M	43		11.5	4
2	M	44	Attention deficit disorder	11.6	1
3	M	50	Bronchial asthma, hypertension	11.7	3
4	M	35	IgA-nephritis	12.7	1
5	M	35	Allergic rhinoconjunctivitis, bronchial asthma, chronic urticaria	12.7	2
6	F	43	Hypertension	13.4	1
7	M	71		13.7	3
8	F	52	Hypertension	13.8	4
9	M	25	Hypertension, psoriasis	13.9	3
10	F	41		15.2	1
11	M	45		17.3	4
12	M	71	Systemic mastocytosis, myelodysplastic syndrome	18.4	3
13	F	47	Hypertension	18.8	4
14	F	70	Xerosis cutis	19.1	1
15	M	32	Food allergy	19.2	4
16	M	37		19.47	4
17	M	55		20.3	2
18	F	65		20.6	4
19	M	16	Hypertension, atopic dermatitis	20.9	2
20	F	59		22	4
21	F	53		24	4
22	F	73		25.3	2
23	F	77		27.9	4
24	M	34	Hypertension, hyperuricemia	38	4
25	M	32	Chronic urticaria, hypertension, allergic rhinoconjunctivitis, bronchial asthma	38.7	4
26	F	60	Hypertension	40.9	3
27	F	58		48.5	3
28	F	57	Systemic mastocytosis, M. Cushing, hypertension, adenoma hypophysis, osteoporosis, adipositas, diabetes mellitus	50.1	4
29	M	63	Cutaneous mastocytosis, atopic dermatitis	68.4	2
30	M	70	Systemic mastocytosis	140	4

old (Fig. 1B). These data suggested that not only elevated tryptase concentrations but also increasing age represents important individual risk factor for severe anaphylactic reactions to hymenoptera stings.

Influence of age or gender on the basal serum tryptase level

We observed a general increment in tryptase level in elderly people ($P = 0.0001$) on average, when all data, including those one exceeding the cut-off of $11.4 \mu\text{g/l}$, were analyzed (Fig. 2A). Most importantly, a continuous increase in tryptase concentrations even below the cut-off ($11.4 \mu\text{g/l}$) with increasing age ($P = 0.0026$) was also detectable (Fig. 2B). Gender did not seem to influence the tryptase level (two-sample t -test; $P = 0.8$).

Discussion

It is of great clinical importance to identify those patients at risk for developing life-threatening anaphylactic reactions (1,

3). To date, three major risk factors have been identified: (i) mastocytosis with or without elevated serum tryptase levels, (ii) elevated serum tryptase levels in patients without mastocytosis, and (iii) elderly people.

Schwartz et al. and others noticed that patients with severe anaphylactic reactions displayed higher tryptase levels even before sting challenge compared to nonreactors (11, 18–21). Ludolph-Hauser et al. (5) demonstrated the frequent occurrence of constitutively elevated tryptase levels selectively in these patients with cutaneous mastocytosis who had suffered from systemic hymenoptera sting reaction. Interestingly, tryptase levels also seem to function as an individual risk factor in nonmastocytosis patients. Recent studies have demonstrated that basal serum tryptase levels (cut-off: $11.4 \mu\text{g/l}$) were elevated in approximately 10% of venom-allergic patients and these increased levels correlated significantly with sting reaction severity (4, 11, 22).

In this study, we found that basal serum tryptase levels (cut-off: $11.4 \mu\text{g/l}$) were elevated in 10.9% of venom-allergic patients and this correlated significantly with sting reaction

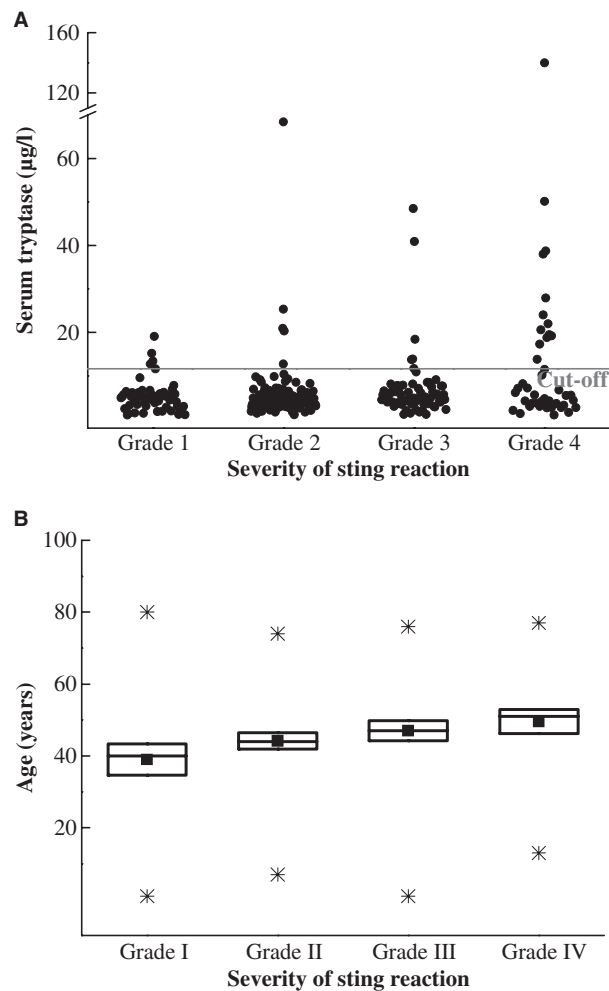


Figure 1 Basal serum tryptase levels and age as a factor in the risk assessment for severe anaphylactic sting reaction. (A) The risk for severe sting reaction increased significantly with rising serum tryptase levels [univariate logistic regression of the data yielded $P = 0.0003$ for odds ratio (OR) level 1 vs level 4]. (B) The risk for severe sting reactions increased significantly with advanced age [univariate logistic regression of the data yielded $P = 0.001$ for odds ratio (OR) level 1 vs level 4].

severity and age of the patients. These data are in line with reports from Kucharewicz et al. (11) showing augmented tryptase levels in 11% of allergic patients. Of interest is another study by Sturm et al. (22) In this presentation, even patients with tryptase levels between 11.4 and 6.6 µg/l also displayed an increased risk for severe sting reactions. In our study, 13 patients with tryptase levels between 11.4 and 6.6 µg/l had grade III reactions, and five patients reported of grade IV reactions. These findings suggest that even lower cut-off levels (>6.6 µg/l) of serum tryptase should be further discussed.

In our study, cutaneous or systemic mastocytosis according to the WHO guidelines could be diagnosed in just 7.5% of the patients with elevated serum tryptase level. Mastocytosis

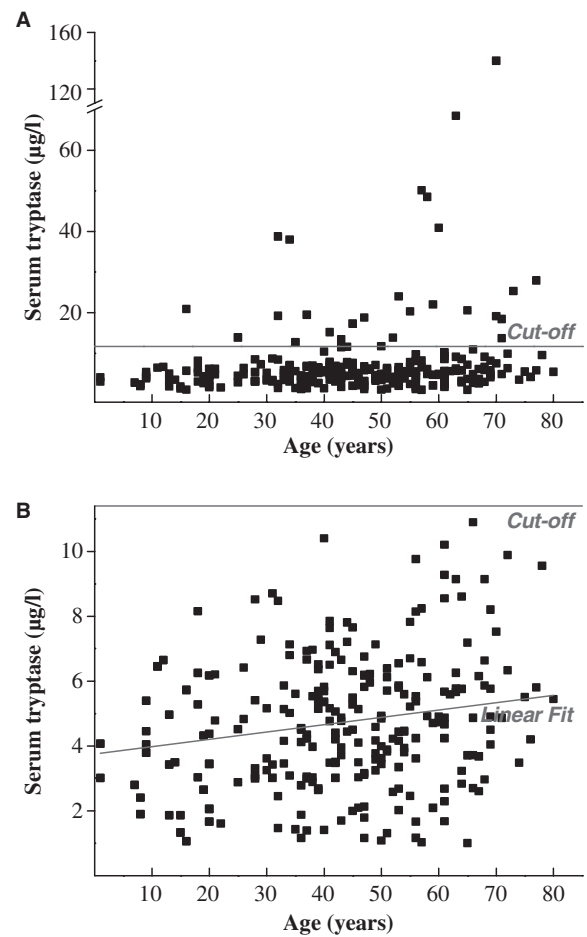


Figure 2 Impact of age on the basal serum tryptase levels. (A) A general increment in tryptase level in elderly people ($P = 0.0001$) and (B) a continuous increase in tryptase concentrations also below the cut-off (11.4 µg/l) with increasing age ($P = 0.0026$).

sis is a rare disease, seen in approximately every 1000–8000 dermatological outpatient visits (23). It is difficult to diagnose, and skin changes are often missed because of faint cutaneous manifestations (7). In our department, bone marrow biopsy was routinely offered to patients with serum tryptase levels above 15 µg/l. It is possible that in some patients with serum tryptase levels between 11.4 and 15 µg/l, systemic involvement may have been overlooked. These patients might be underdiagnosed with 'indolent mastocytosis'. Anyway, serum tryptase can be in normal ranges in patients with mastocytosis, and serum tryptase and even bone marrow biopsy without pathological findings do not exclude mastocytosis (24). Our findings are in line with other reports showing that elevated tryptase concentrations correlate with sting severity, however, without obligatory finding of mastocytosis in those patients (11).

The causes for constitutively raised tryptase concentrations in certain patients are not clear. Other reasons beside mastocytosis could be acute myelocytic leukemia, various myelodysplastic syndromes, hypereosinophilic syndrome associated

with the FLP1L1-PDGFR mutation, end-stage renal failure, and treatment of onchocerciasis (25).

It has been known for a long time that elderly people suffer from increased severity of sting reactions (3, 12, 13). The reason behind this phenomenon remains unclear, though an imbalanced cardiovascular system in those patients has been brought under suspicion (6).

Our data identify increased serum tryptase levels in elderly people as an individual risk factor for severe allergic sting reaction. We could evidence a significant increase in serum tryptase with increasing age, and these elevated levels correlate significantly with the severity of sting reactions. Thus, we could identify serum tryptase as a key factor for the risk assessment especially in elderly people. Underlying causes for these increased basal serum tryptase levels in elderly people

could be, at least partially, responsible for increased severity of allergic reaction observed in those patients. Clarification of the mechanisms, which lead to these increased serum tryptase levels in elderly, needs further investigation. To this end, it could be postulated that various myelodysplastic syndromes that are often overlooked at start could play a role especially in elderly patients and should therefore receive special consideration.

However, as those patients with increased serum tryptase levels are beyond doubt at increased risk for life-threatening anaphylactic reactions, we propose that especially elderly patients with increased tryptase levels should be treated with increased venom doses during immunotherapy, and a life-long continuation may also be considered as recommended for mastocytosis patients with mastocytosis (26).

References

- Muller U, Mosbech H. Position paper: immunotherapy with hymenoptera venoms. (EAACI) The European Academy of Allergology and Clinical Immunology. *Allergy* 1993;**48**:37–46.
- Clark S, Camargo CA Jr. Epidemiology of anaphylaxis. *Immunol Allergy Clin North Am* 2007;**27**:145–163. v.
- Bilo BM, Bonifazi F. Epidemiology of insect-venom anaphylaxis. *Curr Opin Allergy Clin Immunol* 2008;**8**:330–337.
- Haeblerli G, Bronnimann M, Hunziker T, Muller U. Elevated basal serum tryptase and hymenoptera venom allergy: relation to severity of sting reactions and to safety and efficacy of venom immunotherapy. *Clin Exp Allergy* 2003;**33**:1216–1220.
- Ludolph-Hauser D, Rueff F, Fries C, Schopf P, Przybilla B. Constitutively raised serum concentrations of mast-cell tryptase and severe anaphylactic reactions to Hymenoptera stings. *Lancet* 2001;**357**:361–362.
- Mueller UR. Cardiovascular disease and anaphylaxis. *Curr Opin Allergy Clin Immunol* 2007;**7**:337–341.
- Biedermann T, Rueff F, Sander CA, Przybilla B. Mastocytosis associated with severe wasp sting anaphylaxis detected by elevated serum mast cell tryptase levels. *Br J Dermatol* 1999;**141**:1110–1112.
- Brockow K, Jofer C, Behrendt H, Ring J. Anaphylaxis in patients with mastocytosis: a study on history, clinical features and risk factors in 120 patients. *Allergy* 2008;**63**:226–232.
- Schwartz LB, Sakai K, Bradford TR, Ren SL, Zweiman B, Worobec AS et al. The alpha form of human tryptase is the predominant type present in blood at baseline in normal subjects and is elevated in those with systemic mastocytosis. *J Clin Invest* 1995;**96**:2702–2710.
- Schwartz LB, Yunginger JW, Miller J, Bokhari R, Dull D. Time course of appearance and disappearance of human mast cell tryptase in the circulation after anaphylaxis. *J Clin Invest* 1989;**83**:1551–1555.
- Kucharewicz I, Bodzenta-Lukaszyk A, Szymanski W, Mroczko B, Szmitkowski M. Basal serum tryptase level correlates with severity of hymenoptera sting and age. *J Invest Allergol Clin Immunol* 2007;**17**:65–69.
- Lockey RF, Turkeltaub PC, Baird-Warren IA, Olive CA, Olive ES, Peppe BC et al. The Hymenoptera venom study I, 1979–1982: demographics and history-sting data. *J Allergy Clin Immunol* 1988;**82**(3 Pt 1):370–381.
- Lantner R, Reisman RE. Clinical and immunologic features and subsequent course of patients with severe insect-sting anaphylaxis. *J Allergy Clin Immunol* 1989;**84**(6 Pt 1):900–906.
- Przybilla B, Ring J, Grieshammer B. Association of features of atopy and diagnostic parameters in hymenoptera venom allergy. *Allergy* 1991;**46**:570–576.
- Mueller HL. Diagnosis and treatment of insect sensitivity. *J Asthma Res* 1966;**3**:331–333.
- Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. *Blood* 2009;**114**:937–951. Epub 2009 Apr 8.
- Vardiman JW, Harris NL, Brunning RD. The World Health Organization (WHO) classification of the myeloid neoplasms. *Blood* 2002;**100**:2292–2302.
- Dugas-Breit S, Przybilla B, Schopf P, Rueff F. Possible circadian variation of serum mast cell tryptase concentration. *Allergy* 2005;**60**:689–692.
- Schwartz LB. Tryptase: a clinical indicator of mast cell-dependent events. *Allergy Proc* 1994;**15**:119–123.
- Schwartz LB, Bradford TR, Rouse C, Irani AM, Rasp G, Vanderzwan JK et al. Development of a new, more sensitive immunoassay for human tryptase: use in systemic anaphylaxis. *J Clin Immunol* 1994;**14**:190–204.
- Van der Linden PWG, Hack CE, Poortman J, Viviekipf YC, Struyvenberg A, Vanderzwan JK. Insect-sting challenge in 138 patients—relation between clinical severity of anaphylaxis and mast-cell activation. *J Allergy Clin Immunol* 1992;**90**:110–118.
- Sturm G, Wiednig M, Strele A, Schuster C, Aberrer W. Baseline serum tryptase levels and the severity of sting reactions in Hymenoptera venom allergy. Abstract book of the XXV Congress of EAACI. Vienna, 2006:48 [Oral Communication].
- Sagher F, Even-Paz Z. Mastocytosis and the mast cell. Chicago: Year Book Medical Publishers, 1967.
- Ispas L, Henriksen RA, Metzger WJ. The many faces of systemic mastocytosis. *Ann Allergy Asthma Immunol* 2001;**87**:6–14.
- Schwartz LB. Diagnostic value of tryptase in anaphylaxis and mastocytosis. *Immunol Allergy Clin North Am* 2006;**26**:451–463.
- Bonifazi F, Jutel M, Bilo BM, Birnbaum J, Muller U. Prevention and treatment of hymenoptera venom allergy: guidelines for clinical practice. *Allergy* 2005;**60**:1459–1470.