

Importance of serum basal tryptase levels in children with insect venom allergy

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Abstract

Background: The importance of serum basal tryptase (sBT) levels on patients with venom allergy is highlighted in recent adulthood studies. The aim of this study was to evaluate the sBT levels of venom-allergic children with varying severity of clinical reactions. We also aimed to document the association between sBT levels and severe systemic reactions (SR).

Methods: Serum basal tryptase levels were estimated by UniCAP (Pharmacia & Upjohn, Uppsala, Sweden). Children who suffered from large local reaction (LLR) or SR after insect stings were included along with healthy control subjects without a history of any local or SR after insect stings.

Results: A total of 128 children (55 with SR, 18 with LLR, and 55 age and sex-matched control subjects) with a median age of 8.9 years (range 3.2–17.4) were enrolled. Severe SR was encountered in 24 (44%) patients with SRs. The median level of sBT in children with SRs (median, interquartile range) [4.2 µg/l (3.6–4.9)] was significantly higher than in children with LLRs [3.1 µg/l (2.5–4.0)] and healthy control subjects [2.9 µg/l (2.3–3.4)] ($P < 0.001$). Logistic regression analysis revealed sBT ≥ 4.8 µg/l as a significant risk factor for severe SR (5.7 [1.5–21.4]; $P = 0.01$) in children with venom allergy.

Conclusions: Our results indicate that sBT levels are associated with a higher risk of severe SR in children with insect venom hypersensitivity. Determination of sBT levels may help clinicians to identify patients under risk of severe SRs and optimal and timely use of therapeutic interventions in children with venom allergy.

Hymenoptera stings are frequently encountered in the population and may lead to different clinical reactions ranging from local reactions to severe and life-threatening systemic anaphylactic reactions in sensitized individuals (1). Reactions in children are generally reported as less severe than adults and predominantly isolated cutaneous symptoms without respiratory or cardiovascular involvement (2). Although the results of previous studies including predominantly adult patients have established several risk factors associated with the severity of reactions, it is not still possible to accurately predict the type and severity of future reactions (3, 4).

Along with the established risk factors such as older age, accompanying asthma, vespid venom allergy, underlying cardiovascular diseases and use of angiotensin-converting

enzyme inhibitor and beta blocker medications, recent studies have determined increased serum basal tryptase (sBT) levels particularly in patients with a history of severe systemic reaction (SR) (3, 5–9). Moreover, such patients are recommended to undergo further investigations regarding underlying mastocytosis or monoclonal mast cell activation syndromes (5–7). Increased sBT levels have also been established as a risk factor for potential side-effects during venom immunotherapy (10). However, there is a lack of data regarding the role of sBT levels in the type and severity of clinical reaction in the pediatric literature.

The aim of this study was to evaluate the sBT levels of venom-allergic children with different type and severity of clinical reactions along with children without insect venom allergy.

Materials and methods

Patients

Children who were referred to the pediatric allergy outpatient department with a history of large local reaction (LLR) or systemic reaction (SR) after hymenoptera sting from January 2010 to October 2011 were consecutively enrolled and analyzed along with control subjects who admitted to outpatient department of our unit with various symptoms of suspected atopic diseases such as asthma, rhinitis, dermatitis, and urticaria. None of the patients in the control group had a documented history of any local or systemic reaction after insect stings or a concurrent illness that would cause a change in tryptase value. All children and their parents were interviewed, and a questionnaire including information about insect sting history, medication, and demographic data was performed. The children with a clinical history of an LLR after hymenoptera stings without any evidence of previous systemic reactions were enrolled in the LLR group. The index sting, to which all other information was referred, was defined as that field sting that had resulted in the hitherto most severe SR (in SR group) or LLR (in LLR group) according to the individual patient's history. If a patient had several sting reactions with a comparable degree of severity and that were the most severe reactions overall, the first, most severe sting was taken as the index sting (7). Picture cards of insects (wasps and bees) were displayed to children and parents who had noticed the sting in order to determine the implicated insect. For all sting reactions, the presence of detailed systemic symptoms was queried and recorded. The study had approval from the Institutional Review Board of Hacettepe University, and written informed consent forms were obtained from the children and their parents.

Diagnosis of venom allergy

Diagnosis of hymenoptera venom allergy was made by using guidelines published by the European Academy of Allergy and Clinical Immunology (EAACI) and the American Academy of Allergy, Asthma and Immunology (1, 11). Children with a history of previous systemic sting reaction were diagnosed as having venom allergy after confirming the sensitization by a skin test reaction (prick and intradermal tests with *Vespula* (wasp) and *Apis mellifera* (bee) venoms at least 2 weeks after the reaction) and/or the detection of specific IgE (sIgE) to the implicated type of insect as shown by increased titers of sIgE (>0.35 kU/l) measured by the Pharmacia CAP System method (Uppsala, Sweden) (1). Skin tests (prick or intradermal) were not performed in children with a history of LLRs and children without any reaction after stings (12, 13).

Large local reaction is defined as edema and erythema at the site of the sting exceeding a diameter of 10 cm which lasts longer than 24 h (1).

Severity of systemic reactions

The severity of SRs was graded according to the system proposed by Ring and Messmer who described 4 different grades

(4, 14). A mild SR was defined as a Grade I or II reaction, whereas a severe SR as Grade III or IV.

Study measurements

Skin tests

Skin tests were performed with bee and wasp venom (*Apis Mellifera*, *Vespula*, Stallergenes, France) in children with systemic reactions (1). Skin prick test was performed with 1 µg/ml of venom concentration; if negative, then intradermal injections with concentration of tenfold dilutions ranging from 0.001–1 µg/ml and 0.02 ml of venom were done into the volar surface of the forearm (1). Skin prick test was considered positive if a mean diameter of the resulting wheal was at least 3 mm after 15 min compared to the negative control. Intradermal test was read as positive at a mean wheal diameter of at least 5 mm with erythema with concentration of 1 µg/ml or less. Histamine and saline were used as positive and negative controls, respectively. The test was terminated at the positive concentration.

All participants were also skin-prick-tested for common aeroallergens for our region, including *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Blattella germanica*, cat, horse, dog, *Alternaria alternata*, *Aspergillus fumigatus*, *Penicillium mixture* (*P. digitatum*, *P. expansum*, and *P. notatum*), *Cladosporium herbarum*, *Phleum pratense*, *Poa pratensis*, *Dactylis glomerata*, *Lolium perenne*, *Festuca pratensis*, *Avena sativa*, *Cynodon dactylon*, *Parietaria judaica*, *Artemisia vulgaris*, *Plantago*, *Chenopodium mixture*, *Salsola kali*, *Salix caprea*, *Ulmus campestris*, *Quercus robur*, hazel, *Betula alba*, *Populus alba*, *Platanus vulgaris*, and *Olea europaea*, and histamine (10 mg/ml of histamine phosphate) as positive and 0.9% sterile saline as negative controls. (15). The tests were applied on the volar surface of both forearms and recorded after 15 min, and considered as positive if the mean wheal diameter was ≥ 3 mm compared to the negative control. 'Aeroallergen sensitization' was defined as 'having at least one positive skin test response' to one of the aeroallergens tested.

In vitro tests

Serum allergen sIgE level to bee and wasp venom and total IgE (tIgE) levels were measured in all children with the ImmunoCAP system (PhadiaAB, Uppsala, Sweden) according to the manufacturer's instructions. Specific IgE levels were measured at least 2 weeks after the sting reaction (1). The measuring range of the ImmunoCAP system is 0.35–100 kU/l. The results were graded as class 0, 1, 2, 3, 4, 5, and 6 with sIgE levels of <0.35 , 0.35–0.7, 0.7–3.5, 3.5–17.5, 17.5–50, 50–100, and >100 kU/l, respectively. Results >0.35 kU/l were considered as positive.

Eosinophil counts were performed at least 2 weeks after the reaction and determined from Coulter Counter (Beckman Coulter, Fullerton, CA, USA) leukocyte measurements.

Serum tryptase levels

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. Serum tryptase levels were

estimated by UniCAP (System ImmunoCAP Tryptase®, Pharmacia & Upjohn). According to the manufacturer, the interassay variability for tryptase levels between <1 and 100 µg/l is <5% and the upper 95th percentile for healthy nonallergic subjects is 11.4 µg/l. A sBT of ≥ 11.4 µg/l was considered increased.

Diagnosis of asthma

In children with a history of symptoms suggestive of asthma, the diagnosis was made in the presence of reversible airway obstruction as defined by at least a 12% improvement in FEV₁ following bronchodilator administration in children capable of doing spirometry or improvement in asthma symptoms after inhaled steroid or leukotriene modifier agent therapy (16).

Diagnosis of allergic rhinitis

Allergic rhinitis is clinically defined by the occurrence of symptoms such as rhinorrhoea, nasal obstruction, nasal itching, and sneezing upon exposure of sensitized allergen for at least a minimum duration of 2 years (17).

Pediatric allergy specialists made diagnoses of accompanying diseases.

Statistical Analysis

The PASW Statistics software package version 18.0 (SPSS Inc, Chicago, Illinois, USA) was used for all calculations. Descriptive data for categorical and numerical variables were expressed as frequencies and means with standard deviations for normally distributed variables or medians with quartiles in parenthesis for the non-normally distributed variables. Group comparisons were established by using Mann–Whitney U-test or ANOVA on ranks as appropriate and the chi-square test or Fisher test for categorical variables. The role of sBT levels in predicting clinical reactivity was analyzed using logistic regression modeling. The diagnostic

performances of sBT levels to predict SR and severe SR were determined by receiver operating characteristics (ROC) curve analysis. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for each selected cutoff points. A *P* level <0.05 was considered significant.

Results

Seventy-three children (55 with SR and 18 with LLR) with insect venom allergy were enrolled along with 55 age- and sex-matched control subjects. Male gender was more frequent in children with LLRs (*P* = 0.032). Total IgE levels of children with SR were significantly higher than that of children in control group [160 (76–418) kU/l vs 42 (16–96) kU/l; *P* < 0.001]. Demographic and laboratory features of study population are presented in Table 1.

The median level of sBT in children with SRs (median, interquartile range) [4.2 µg/l (3.6–4.9)] was significantly higher than in children with LLRs [3.1 µg/l (2.5–4.0)] and control subjects [2.9 µg/l (2.3–3.4)] (*P* < 0.001) (Figs 1 and 2 A). Of the 128 patients, sBT level was increased only in one child (14.5 µg/l) in the SR group and screening for mastocytosis was negative for him.

When reactions of children with SRs were graded according to the classification of Ring-Messmer (Table 2), most of them were Grades II and III. Data of children are presented in Table 3.

Analysis using ROC curves identified the diagnostic role of sBT levels in predicting severe SR risk in the group of children with SRs (AUC: 0.66, 95% CI: 0.51–0.81, *P* = 0.046). When analyzed for the best cutoff value with the highest combined sensitivity and specificity, a cutoff value of 4.8 µg/l for sBT level was obtained with sensitivity, specificity, PPV, and NPV of 45.8%, 87.1%, 73.3%, and 67.5%, respectively. The result of univariate logistic regression analysis revealed that a sBT level ≥ 4.8 µg/l was a significant risk factor for severe SR in

Table 1 Characteristics of the study group

	SR	LLR	NR
<i>N</i>	55	18	55
Current age*	10.1 (7.5–12.4)	8.4 (6.0–13.0)	8.1 (5.7–11.4)
Age at index sting reaction*	8.6 (6.0–11.1)	8.4 (5.3–11.1)	–
Gender (M/F), <i>n</i> (%)	35/20 (64/36)	16/2 (89/11)†	30/25 (55/45)
Implicated insect (Bee/Wasp), <i>n</i> (%)	7/48 (13/87)	1/17 (6/94)	–
Concomitant asthma, <i>n</i> (%)	10 (18)	3 (17)	6 (11)
Concomitant allergic rhinitis, <i>n</i> (%)	9 (16)	4 (22)	7 (13)
Aeroallergen sensitization, <i>n</i> (%)	16 (29)	4 (24)	10 (18)
Family history of atopic disease, <i>n</i> (%)	18 (33)	6 (33)	19 (35)
slgE for implicated insect*	6.8 (3.4–27.1)	5.3 (0.5–11.2)	–
Total IgE (kU/l)*	160 (76–418)	91 (28–321)	42 (16–96)‡
Eosinophil (%)	3.2 (1.8–4.1)	1.9 (1.2–4.5)	2.0 (1.2–4.9)
Eosinophil count (/mm ³)*	200 (100–300)	150 (100–325)	200 (100–300)
sBT (µg/l)*	4.2 (3.6–4.9)‡	3.1 (2.5–4.0)	2.9 (2.3–3.4)
sBT (µg/l), range	1.3–14.5	1.1–5.3	1.0–6.1

SR, systemic reaction; LLR, large local reaction; NR, no reaction; sBT, serum basal tryptase.

*Median (interquartile range); †*P* < 0.05; ‡*P* < 0.001.

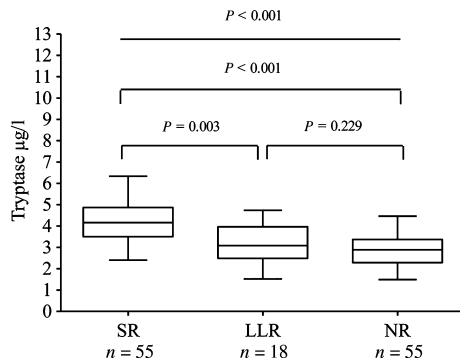


Figure 1 Serum tryptase levels of study population.

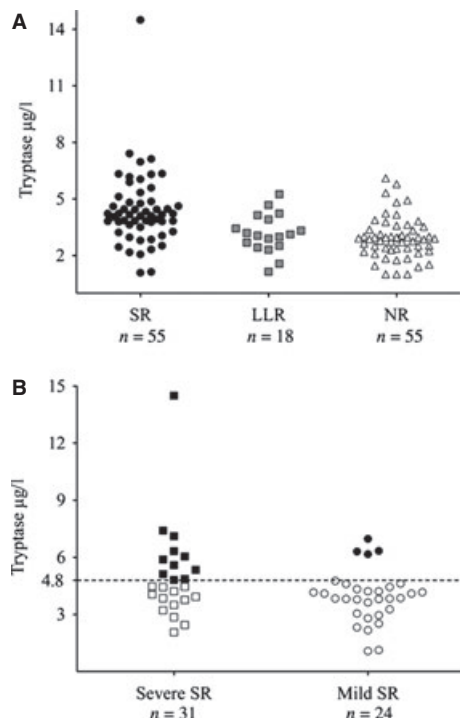


Figure 2 (A) Distribution of serum basal tryptase levels of study population. (B) Distribution of serum basal tryptase levels of children with systemic reactions.

venom-allergic children with SRs (OR[95% CI]; 5.7 [1.5–21.4]; $P = 0.01$). The prevalence of children with sBT ≥ 4.8 µg/l were significantly higher in severe SR group when compared to children with mild SRs ($P = 0.007$) (Table 3 and Fig. 2B).

The predicted probabilities for a severe SR at a given sBT level that were calculated by logistic regression analysis are presented in Fig. 3. The curve of predicted probabilities for sBT determines that severe SR probability increases at levels greater than 5 µg/l.

Discussion

In the present study, we found out that sBT levels of children with SRs were higher than those of children with LLRs or

Table 2 Frequency and classification of systemic reactions ($n = 55$) modified according to Ring and Messmer

Severity	Classification	Symptoms	Frequency, $n(\%)$
Mild	Grade I	Generalized skin symptoms (e.g., flush, generalized urticaria, angioedema)	3 (5.5)
	Grade II	Mild-to-moderate pulmonary, cardiovascular, and/or gastrointestinal symptoms	22 (40)
Severe	Grade III	Anaphylactic shock, loss of consciousness	23 (41.8)
	Grade IV	Cardiac arrest, apnea	7 (12.7)

Table 3 Demographic and laboratory data of children with SRs

	Mild SR	Severe SR
N	31	24
Age*	10.6 (7.6–12.4)	9.6 (7.1–12.7)
Gender (M/F), $n(\%)$	18/13 (58/42)	17/7 (71/29)
Age at last SR*	8.5 (6.1–10.8)	9.0 (5.3–11.7)
Implicated insect (Bee/Wasp), $n(\%)$	3/28	4/20
Aeroallergen sensitization, $n(\%)$	10 (32)	6 (25)
Concomitant asthma, $n(\%)$	8 (26)	5 (22)
Concomitant allergic rhinitis, $n(\%)$	6 (19)	4 (17)
sIgE for implicated insect*	6.6 (2.7–18.1)	13.6 (4.0–40.3)
Total IgE*	136 (66–375)	201 (106–470)
Eosinophil (%)*	2.9 (1.5–4.1)	3.2 (1.9–4.4)
Eosinophil (/mm ³)*	200 (100–300)	200 (148–375)
sBT (µg/l)*	4.0 (3.2–4.5)	4.5 (3.8–5.8) [†]
Patients with sBT ≥ 4.8 µg/l, $n(\%)$	4 (13)	11 (46) [†]

*median (IQR); [†] $P < 0.05$.

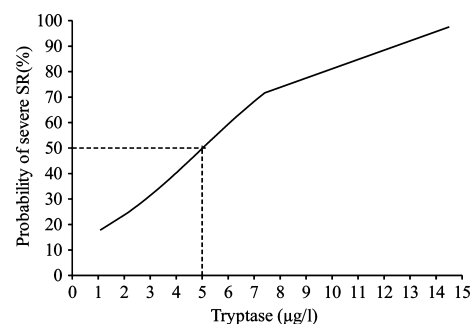


Figure 3 Predicted risk for severe systemic reaction according to serum basal tryptase levels.

children with no reactions after insect stings. Moreover, we determined the role of sBT levels in predicting severe SR risk in children with venom allergy.

The results of the studies from children with venom allergy usually report a milder clinical presentation than adults with predominantly isolated cutaneous symptoms and rare severe respiratory or cardiovascular symptoms (2, 18, 19). However, approximately half of the cases in our study population with a history of SR had encountered severe reactions and this is a relatively high prevalence when compared to previous studies. Our center is the largest referral center in Turkey and referral of predominantly severe cases might have contributed to this conclusion. Further multicenter studies with a larger group of children are required for better understanding of the natural course of the condition.

Venom-induced systemic reactions have been rarely reported in patients with mastocytosis, and this association was considered as co-incidental till the end of 1990s (20, 21). However, this opinion has changed after documentation of the relationship between sBT levels and severe SR by Ludolph-Hauser et al. in 2001 (6). This association was reconfirmed in two subsequent studies by Haerberli et al. and Kucharewicz et al. (22, 23) in different populations consisted a total of 368 predominantly adult patients. A significant increase in SR severity and sBT levels by age was also determined, and they have recommended longer durations of immunotherapy – maybe lifetime – for elder patients with high sBT levels (23). Similar conclusions and recommendations were repeated in the study of Blum et al. (24) which they have retrospectively analyzed the data of 758 adult patients with venom allergy in 2011. The results of the observational multicenter study by EAACI interest group on insect venom hypersensitivity in 962 adult patients who had a history of anaphylaxis/SR concluded that higher sBT levels were significantly associated with severe anaphylaxis along with other factors such as vespid venom allergy, older age, male sex, angiotensin-converting enzyme inhibitor medication, and one or more preceding field stings with a less severe SR (7). Major difference in the results of this study from the previous ones was that minor increases ($>5 \mu\text{g/l}$) in sBT levels significantly increased the risk of severe SR even in normally accepted ranges ($<11.4 \mu\text{g/l}$). A sBT level higher than $6.6 \mu\text{g/l}$ has been reported to increase severe SR risk in the study of Guenova et al. (9).

Several childhood studies have investigated the natural course of serum basal tryptase levels, and they are found significantly higher in younger infants compared with older ones along with a gradual decrease before reaching levels similar to adults (25, 26). Moreover, a significant and continual increase was also determined by age (27–29). The cutoff value for sBT levels ($4.8 \mu\text{g/l}$) that were determined by analyzing the data of children with a history of SR/anaphylaxis is similar to the value ($5 \mu\text{g/l}$) that was determined by Rueff et al. (7) for adult patients. Our findings may encourage physicians to initiate venom immunotherapy particularly in children with sBT levels higher than $4.8 \mu\text{g/l}$ and with a history of mild SRs after stings. Further studies with larger study populations may be required to reconfirm this finding.

Although the study groups were significantly different in terms of median sBT levels and prevalence of children with high ($>4.8 \mu\text{g/l}$) sBT levels, the differentiation value of the obtained cutoff level was not strong enough for an individual as there was significant overlap between groups. This issue may be related to predominant referral of severe cases due to the tertiary nature of our center, and it may limit the use of cutoff values in daily clinical settings. This limitation can be overcome by the associated usage of further laboratory and/or clinical findings along with sBT levels.

There are several strengths of our study. First of all, our study groups consisted of children with reactions of varying type and severity along with control subjects without venom allergy. It has provided the opportunity not only to precisely document sBT levels in children with varying type and severity of clinical reactions after hymenoptera stings, but also to increase the generalization of our results to children with venom allergy.

Our study is one of the very few childhood studies that investigate the association between sBT levels and the severity of clinical reactivity. Our results also provided unique demographic and laboratory data particularly about children with LLRs which can be defined as a relatively rare investigated subgroup of patients with venom allergy. Its prospective and controlled design along with a fairly large number of participants are other superiorities.

In conclusion, our results indicate that sBT levels are associated with a higher risk of severe SR in children with insect venom hypersensitivity. Determination of sBT levels in children with venom allergy may help clinicians to detect and inform the patients under risk of severe SRs and optimal and timely use of therapeutic interventions in children.

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Authors' contributions

A Tuncer had primary responsibility for protocol development and analytic framework of the study, outcome assessment, and manuscript preparation. ST Yavuz participated in the development of the protocol and analytic framework for the study, had primary responsibility for review of files, patient screening, enrollment, and data entry, and prepared the manuscript with A Tuncer. UM Sahiner, B Buyuktiryaki, EA Yilmaz and OU Soyer had contributed to data generation, patient screening, data analysis and prepared the manuscript with A Tuncer. C Sackesen and B Sekerel had contributed to protocol development, data analysis, and preparation and revision of the manuscript.

Conflict of interest

There is no potential conflict of interest. This study was presented as a thematic poster presentation at the EAACI Congress 2012 16–20 June, Geneva, Switzerland, and awarded with an abstract prize.

References

1. Bilo BM, Rueff F, Mosbech H, Bonifazi F, Oude-Elberink JN. Diagnosis of Hymenoptera venom allergy. *Allergy* 2005;**60**: 1339–1349.
2. Schuberth KC, Lichtenstein LM, Kagey-Sobotka A, Szklo M, Kwitrovich KA, Valentine MD. An epidemiologic study of insect allergy in children. I. Characteristics of the disease. *J Pediatr* 1982;**100**:546–551.
3. Bilo MB, Bonifazi F. The natural history and epidemiology of insect venom allergy: clinical implications. *Clin Exp Allergy* 2009;**39**:1467–1476.
4. Przybilla B, Rueff F. Hymenoptera venom allergy. *J Dtsch Dermatol Ges* 2010;**8**: 114–127; quiz 128–130.
5. Bonadonna P, Perbellini O, Passalacqua G, Caruso B, Colarossi S, Dal Fior D, et al. Clonal mast cell disorders in patients with systemic reactions to Hymenoptera stings and increased serum tryptase levels. *J Allergy Clin Immunol* 2009;**123**:680–686.
6. 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.
7. Rueff F, Przybilla B, Bilo MB, Muller U, Scheipl F, Aberer W, et al. Predictors of severe systemic anaphylactic reactions in patients with Hymenoptera venom allergy: importance of baseline serum tryptase—a study of the European Academy of Allergology and Clinical Immunology Interest Group on Insect Venom Hypersensitivity. *J Allergy Clin Immunol* 2009;**124**:1047–1054.
8. Settapani GA, Chafee FH, Klein DE, Boyd GK, Sturam JH, Freye HB. Anaphylactic reactions to Hymenoptera stings in asthmatic patients. *Clin Allergy* 1980;**10**:659–665.
9. Guenova E, Volz T, Eichner M, Hoetzelnecker W, Caroli U, Griesinger G, et al. Basal serum tryptase as risk assessment for severe Hymenoptera sting reactions in elderly. *Allergy* 2010;**65**:919–923.
10. Rueff F, Przybilla B, Bilo MB, Muller U, Scheipl F, Aberer W, et al. Predictors of side effects during the buildup phase of venom immunotherapy for Hymenoptera venom allergy: the importance of baseline serum tryptase. *J Allergy Clin Immunol* 2010;**126**:105–111 e105.
11. Joint Task Force on Practice Parameters; American Academy of Allergy, Asthma and Immunology; American College of Allergy, Asthma and Immunology; Joint Council of Allergy, Asthma and Immunology. Allergen immunotherapy: a practice parameter second update. *J Allergy Clin Immunol* 2007;**120**:S25–S85.
12. Ellis AK, Day JH. Clinical reactivity to insect stings. *Curr Opin Allergy Clin Immunol* 2005;**5**:349–354.
13. Severino M, Bonadonna P, Passalacqua G. Large local reactions from stinging insects: from epidemiology to management. *Curr Opin Allergy Clin Immunol* 2009;**9**: 334–337.
14. Ring J, Messmer K. Incidence and severity of anaphylactoid reactions to colloid volume substitutes. *Lancet* 1977;**1**:466–469.
15. Sahiner UM, Civelek E, Yavuz ST, Buyuktiryaki AB, Tuncer A, Sekerel BE. Skin prick testing to aeroallergens: what is the optimal panel in children and adolescents in Turkey? *Int Arch Allergy Immunol* 2012;**157**:391–398.
16. From the Global Strategy for Asthma Management and Prevention. (updated 2011). Available from: <http://www.ginasthma.org/>. Last accessed Sep 12, 2012.
17. Bousquet J, Khaltaev N, Cruz AA, Denburg J, Fokkens WJ, Togias A, et al. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen). *Allergy* 2008;**63**(Suppl 86):8–160.
18. 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**:370–381.
19. Chipps BE, Valentine MD, Kagey-Sobotka A, Schuberth KC, Lichtenstein LM. Diagnosis and treatment of anaphylactic reactions to Hymenoptera stings in children. *J Pediatr* 1980;**97**:177–184.
20. Oude Elberink JN, de Monchy JG, Kors JW, van Doormaal JJ, Dubois AE. Fatal anaphylaxis after a yellow jacket sting, despite venom immunotherapy, in two patients with mastocytosis. *J Allergy Clin Immunol* 1997;**99**:153–154.
21. Fricker M, Helbling A, Schwartz L, Muller U. Hymenoptera sting anaphylaxis and urticaria pigmentosa: clinical findings and results of venom immunotherapy in ten patients. *J Allergy Clin Immunol* 1997;**100**:11–15.
22. Haeberli 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.
23. Kucharewicz I, Bodzenta-Lukaszyk A, Szymanski W, Mroczko B, Szmikowski M. Basal serum tryptase level correlates with severity of hymenoptera sting and age. *J Investig Allergol Clin Immunol* 2007;**17**:65–69.
24. Blum S, Gunzinger A, Muller UR, Helbling A. Influence of total and specific IgE, serum tryptase, and age on severity of allergic reactions to Hymenoptera stings. *Allergy* 2011;**66**:222–228.
25. Belhocine W, Ibrahim Z, Grandne V, Buffat C, Robert P, Gras D, et al. Total serum tryptase levels are higher in young infants. *Pediatr Allergy Immunol* 2011;**22**:600–607.
26. Komarow HD, Hu Z, Brittain E, Uzzaman A, Gaskins D, Metcalfe DD. Serum tryptase levels in atopic and nonatopic children. *J Allergy Clin Immunol* 2009;**124**:845–848.
27. Dugas-Breit S, Przybilla B, Dugas M, Arnold A, Pfundstein G, Kuchenhoff H, et al. Serum concentration of baseline mast cell tryptase: evidence for a decline during long-term immunotherapy for Hymenoptera venom allergy. *Clin Exp Allergy* 2010;**40**:643–649.
28. Gonzalez-Quintela A, Vizcaino L, Gude F, Rey J, Meijide L, Fernandez-Merino C, et al. Factors influencing serum total tryptase concentrations in a general adult population. *Clin Chem Lab Med* 2010;**48**:701–706.
29. Schwartz LB, Bradford TR, Rouse C, Irani AM, Rasp G, Van der Zwan JK, et al. Development of a new, more sensitive immunoassay for human tryptase: use in systemic anaphylaxis. *J Clin Immunol* 1994;**14**:190–204.