ORIGINAL ARTICLE

Serum Tryptase Level Is a Better Predictor of Systemic Side Effects Than Prostaglandin D_2 Metabolites During Venom Immunotherapy in Children

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Abstract

Objectives: We performed a prospective study to analyze mast cell mediators as predictors of systemic adverse reactions during rush venom-specific immunotherapy (VIT) in children.
Patients and Methods: Nineteen children aged 5-17 years received VIT with Venomenhal (HALAllergy). We analyzed serum tryptase (CAP, Phadia), plasma prostaglandin (PG) D_2 metabolites (9α,11ß-PGF2), and urine PGD_2 metabolites (9α,11ß-PGF_2, tetranor-PGD-M) using gas chromatography mass spectrometry before and after the rush protocol.
Results: Three boys with high baseline serum tryptase values (>7.76 μg/L) (*P*<.001) and low 9α,11ß-PGF_2 concentrations developed grade III systemic adverse reactions during VIT. Baseline serum tryptase was lowest in children who had a Mueller grade II reaction (1.93 [0.36]) before VIT and highest in children with a Mueller grade III reaction (6.31 [4.80]) (*P*=.029). Repeated measures analysis of variance confirmed that, in children who developed systemic adverse reactions during VIT, serum tryptase was higher both before and after desensitization and increased significantly following the procedure. Analysis of PGD_2 metabolites in the prediction of systemic adverse reactions during VIT was inadequate (sensitivity 67% and specificity 0.53%), whilst prediction based on serum tryptase was accurate.
Conclusions: In children with severe systemic adverse reactions to Hymenoptera sting, the evaluation of baseline tryptase levels should be a standard procedure. Children with *Apis mellifera* venom allergy and baseline tryptase levels higher than 7.75 μg/L are at risk of anaphylaxis during buildup. Lower baseline values of plasma and urinary PGD_2 metabolite concentration in patients with systemic adverse reaction during VIT suggest that prostaglandin catabolism is altered.

Key words: Rush venom immunotherapy. Children. Serum tryptase. 9α,11ß-PGF_2. Tetranor-GD-M. PGD_2 metabolites.

Resumen

Objetivos: Se realizó un estudio prospectivo para analizar los mediadores de los mastocitos como factores predictivos de reacciones adversas sistémicas durante la inmunoterapia rápida específica con veneno en niños.
Pacientes y métodos: Diecinueve niños de entre 5 y 17 años de edad recibieron inmunoterapia con veneno con Venomenhal (HAL Allergy). Se analizaron la triptasa sérica (CAP, Phadia), los metabolitos plasmáticos de la prostaglandina (PG) D_2 (9α,11ß-PGF_2) y los metabolitos urinarios de la PGD_2 (9α,11ß-PGF_2, tetranor-PGD-M), utilizando cromatografía de gases y espectrometría de masas antes y después del protocolo rápido.
Resultados: Durante la inmunoterapia con veneno, 3 niños con valores iniciales altos de triptasa sérica (>7,76 μg/l) (*p*<0,001) y concentraciones bajas de 9α,11ß-PGF_2 desarrollaron reacciones adversas sistémicas de grado III. Los niveles iniciales de triptasa sérica fueron más bajos en los niños que, antes de la inmunoterapia con veneno, experimentaron una reacción de grado II en la escala de Mueller (1,93 [0,36]), y más elevados en los niños con una reacción de grado III en la escala de Mueller (6,31 [4,80]) (*p*=0,029). Los análisis

de la varianza con determinaciones repetidas confirmaron que, en los niños que desarrollaron reacciones adversas sistémicas durante la inmunoterapia con veneno, los niveles de triptasa sérica fueron más elevados tanto antes como después de la desensibilización, y aumentaron de forma significativa tras el procedimiento. El análisis de los metabolitos de la PGD_2 como factor predictivo de reacciones adversas sistémicas durante la inmunoterapia con veneno resultó insuficiente (sensibilidad del 67% y especificidad del 0,53%), mientras que la predicción basada en la triptasa sérica resultó exacta.

Conclusiones: En niños con reacciones adversas sistémicas graves a la picadura de himenópteros, la evaluación de los niveles iniciales de triptasa debería ser un procedimiento habitual. Los niños con alergia al veneno de abeja y niveles iniciales de triptasa superiores a 7,75 μg/l presentan riesgo de anafilaxia durante la acumulación. Los valores iniciales más bajos de concentración de metabolitos plasmáticos y urinarios de la PGD_2 en pacientes con reacciones adversas sistémicas durante la inmunoterapia con veneno indican que el catabolismo de las prostaglandinas está alterado.

Palabras clave: Inmunoterapia rápida con veneno. Niños. Triptasa sérica. 9α,11ß-PGF_2. Tetranor-PGD-M. Metabolitos de la PGD_2.

Introduction

Severe systemic reaction to Hymenoptera sting is a potentially life-threatening event. It is caused by a sudden release of mediators derived from mast cells and basophils upon exposure to venom allergens. Demonstration of a rapid and transient increase in serum tryptase level (active mature ß tryptase) during an allergic reaction reflects massive mast cell activation and confirms the diagnosis of anaphylaxis [1]. Baseline serum mast cell tryptase concentration (inactive α-/ ß-protryptases) reflects a constitutive mast cell load or activity and is considered to be a marker of mast cell clonal disorders (mastocytosis) [2]. Elevated baseline serum tryptase level has recently been shown to predict severe systemic reaction both to Hymenoptera stings and during the buildup phase of venom immunotherapy (VIT) in adults [3,4]. As baseline tryptase level seems to increase continuously with age, more severe anaphylactic reactions are observed in elderly people [5].

The prostaglandin (PG) D2 metabolites—9α,11ß-PGF_2 and tetranor-PGD-M—reflect systemic PGD_2 production and are derived exclusively from mast cells and basophils. These mediators are relatively stable and have proven useful in monitoring asthmatic adults [6,7] and children [8,9]. They have also been investigated in children with atopic eczema/dermatitis using quantification of urine 9α,11ß-PGF_2 [10]. Data on 9α,11ß-PGF_2 urinary excretion as a reliable marker of endogenous production of proinflammatory PGD_2 in anaphylaxis are scant [11]. We present the preliminary results of a similar approach in monitoring systemic adverse reactions during VIT in children sensitized to Hymenoptera venom.

Quantification of eicosanoid production using gas chromatography-negative ion chemical ionization-mass spectrometry (GC-NICI-MS) is considered to be the gold standard for reliable routine quantification of eicosanoid production in vivo [12,13]. Few studies monitor mast cell mediators in children with Hymenoptera venom allergy.

Our objective was to assess the predictive value of mast cell mediators (serum tryptase, plasma and urine 9α,11ß-PGF_2, and urine tetranor–PGD-M) in systemic adverse reactions in children sensitized to Hymenoptera venom who were prospectively recruited to undergo a rush VIT protocol.

Patients and Methods

The study sample comprised 19 children (15 boys) aged 5-17 years (mean [SD], 10.6 [3.6] years) who underwent VIT (10 to *Apis mellifera* venom, 9 to *Vespula* venom). The inclusion criteria were systemic reaction to Hymenoptera sting (Mueller grade II-IV) and confirmed immunoglobulin E(Ig)–-mediated allergy to venom. Three to six weeks after field systemic sting reaction, we performed skin prick tests with *Vespula* species venom extract and *Apis mellifera* venom extract (HALAllergy, The Netherlands) at a concentration 100 μg/mL, intradermal tests with updosing to the maximum concentration of 1 μg/mL, and serum specific IgE (SSIgE) determination (CAP System specific IgE FEIA, Phadia, Uppsala, Sweden). The results were interpreted as described elsewhere [1]. The clinical characteristics of the patients and the results of the assays are presented in Table 1. Children fulfilling the inclusion criteria started an 8-day rush protocol with incremental doses of venom (Venomenhal, HALAllergy) (cumulative dose equal to 226.7 μg) (Table 2). Peripheral venous blood and urine samples were taken twice in order to estimate levels of mast cell mediators at baseline, ie, before the first dose of rush VIT (blood, morning in the fasting state; urine, first morning sample), and after the last injection of the incremental dose (blood, after 5 minutes for 9α,11ß-PGF_2 and 1 hour later for tryptase; urine, within 1-2 hours after the last injection). Blood samples for tryptase were allowed to clot, and serum was separated by centrifugation and stored at –80ºC. Total α- and ß-proforms and mature ß tryptase were measured using a fluoroenzyme immunoassay based on the CAP System (Phadia). The tryptase detection method had a range of 1 to 200 μg/L, while normal values were considered to be below 10 μg/L [14]. In the case of values greater than 10 μg/L, we performed duplicate measurements. According to the manufacturer, the interassay variability for tryptase levels between 1.0 and 100 μg/L is below 5%. Blood samples for 9α,11ß-PGF_2 were immediately centrifuged at 3500 rpm for 10 minutes and 0.5 ng of internal deuterated standard $PGF_{2\alpha}$([2H4] $PGF_{2\alpha}$) (CaymanChemicals, AnnArbor, Michigan, USA) was added to 1 mL of plasma. Internal deuterated standard $PGF_{2\alpha}$ ([2H4] $PGF_{2\alpha}$), was also added to 0.5 mL of urine to correct

Table 1. Clinical Characteristics and Results of Assays With Specific Venom Allergy in Treated Patients

Patient number	1	2	3	4	5	6	7	8	9	10[a]	11	12	13	14	15[b,c]	16[d]	17	18	19
Gender	Girl	Boy	Boy	Boy	Boy	Boy	Boy	Boy	Boy	Boy	Girl	Boy	Boy	Boy	Boy	Girl	Girl	1	1
Age, y	5	6	8	10	11	11	11	12	14	6	6	9	10	10	10	15	15	16	17
Systemic adverse reaction	1	1	1	1	1	1	1	1	1	2	1	2	2	1	1	1	1	1	1
Venom allergy (1, *Vespula*; 2, *Apis mellifera)*	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2
Mueller grade before VIT	4	3	4	4	3	3	4	2	4	3	4	3	3	2	4	4	2	4	2
Atopy (positive SPT result to inhalant allergens)	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Pos	Neg	Neg	Neg	Neg	Pos	Pos	Neg	Neg	Neg
Total IgE, kU_A/L	46	2	126	72.10	80.70	157	308	81	495	80	49	262	489	207	424	374	147	243	202.38
Vespula venom sIgE, kU_A/L	0.75	3.37	2.29	6.63	3.04	1.42	11.30	0.40	1.09	0.34	0.40	0.64	1.92	0	1.75	7.95	1.76	0.43	1.69
Honeybee venom sIgE, kU_A/L	0	1.69	0	1.46	0	0	0	0	101	10.30	23.30	66.30	101	1.81	0.70	10.60	26	56.90	32.99
IDT *Vespula* venom concentration 1.0 μg/mL	10	11	9	7	7	7	9	7	5	0	0	0	2	7	0	0	0	5	0
IDT *Apis mellifera* venom concentration 1.0 μg/mL	0	0	0	0	5	0	4	0	10	0	0	9	8 (0.01 μg/mL)	5	0	3	9	9 (0.1 μg/mL)	7 (0.01 (μg/mL)
Baseline serum tryptase, μg/L	3.52	3.62	2.98	3.44	2.44	1	4.49	1.52	2.16	9.42	2.78	7.76	13.60	1.87	4.52	3.17	2.40	3.10	1.94
Serum tryptase after VIT	2.68	2.94	3.29	3.21	2.79	1.58	3.91	1.70	2.37	29.90	4.10	10.30	16.70	2.68	4.47	3.44	2.57	6.85	2.42
Baseline plasma 9α,11ß-PGF_2 concentration, pg/mL	2.90	3.90	2.60	6.50	7.70	2	0.95	3.50	2.80	2.40	3.40	3.80	6.70	3	14.70	4	17	2	8.50
Plasma 9α.11ß-PGF_2 concentration after VIT	23	7.50	4.30	4.80	2.50	4.10	0.51	6.10	2.30	9.70	16.50	11.80	24.90	5.20	30.90	6.20	4.50	7.30	7.80
Baseline urine 9α.11ß-PGF_2 concentration, ng/mg creatinine	0.81	0.63	0.50	1.39	5.90	0.72	1.13	0.80	0.60	1.10	0.50	0.20	0.53	0.40	0.60	0.63	0.90	0.30	0.40
Urinary 9α,11ß-PGF_2 concentration after VIT	0.27	0.07	0.60	0.48	1	0.31	0.10	0.90	0.40	0.40	0.60	0.20	0.59	1	0.61	0.86	0.50	0.29	0.50
Baseline urine PGDM concentration, ng/mg creatinine	1.24	0.92	0.44	0.66	5.67	0.81	0.14	0.76	0.85	2.56	1.46	0.94	0.73	1.63	1.33	0.25	0.70	0.51	0.67
Urinary PGDM concentration after VIT	0.99	0.11	1.46	0.48	1.15	2.79	0.42	1.05	0.78	4.52	2.27	0.56	5.44	3.40	1.34	0.65	0.65	1.25	0.76

Abbreviations: IDT, intradermal test; Ig, immunoglobulin; PG, prostaglandin; SPT, skin prick test; VIT, venom immunotherapy.
[a]Polysensitization to inhalant allergens. Bronchial asthma, allergic rhinitis.
[b]Patient who could have received specific immunotherapy with both venoms but who was included in the category of patients treated against bee venom allergy, as his parameters were collected during Apis mellifera rush VIT, which was completed before his treatment using Vespula venom.
[c]Positive SPT to *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*. Allergic rhinitis, episodic bronchial asthma.
[d]Positive SPT to *Dermatophagoides farinae*. Mild allergic rhinitis.

for the loss of analyte during sample preparation. All samples were stored at –80º C and assayed within 1 month. 9α,11ß-PGF_2 and tetranor-PGD-M were measured using GC-NICI-MS (model 5896 series II; Hewlett Packard, Palo Alto, California, USA) as described elsewhere [6,15,16]. The diagnostic ions were at m/z 569 and m/z 573 for the internal standard of 9α,11ß-PGF_2, and at m/z 489 and m/z 495 for tetranor-PGD-M. The detection limit was 1 pg/mL in plasma samples and 0.5 ng/mg of creatinine in urine samples.

Three patients were atopic (positive skin prick tests Nexter/Allergopharma with inhalant allergens) (Table 1). On each VIT day, patients were examined to rule out any symptoms of infection. Stable clinical condition and peak expiratory flow over 80% of normal value were verified. None of the patients had a history of recurrent urticaria or any clinical symptoms of urticaria pigmentosa. Renal function was normal. No antihistamines, systemic corticosteroids, or leukotriene antagonists were administered during VIT. No systemic adverse effects of VIT were recorded.

Statistical Analysis

Results were described using standard descriptive statistics (mean [SD], range). Comparison within the group of variables measured at 2 time points was performed using the exact Wilcoxon signed rank test. Variables measured at the same time point were contrasted between the groups using the Mann-Whitney test or Kruskal-Wallis test for more than 2 groups when the grouping variable was nominal or with a Jonckheere-Terpstra test when the grouping variable was ordinal. The strength of correlation between variables measured on at least ordinal level was estimated using the Kendall τ-b coefficient. Changes in mediators during VIT, stratified according to the occurrence of systemic adverse effects, were evaluated using univariate repeated measurements analysis of variance (ANOVA). Statistical significance was set at $P<.05$.

Table 2. Eight-Day Rush Protocol of Venom Immunotherapy Dose Increases

Day of VIT	Venom Extract Concentration, µg/mL	Daily Doses of Venom mL	Cumulative Daily Dose, µg
1	0.0001	0.1+0.2+0.4+0.8	0.00015
2	0.001	0.1+0.2+0.4+0.8	0.0015
3	0.01	0.1+0.2+0.4+0.8	0.015
4	0.1	0.1+0.2+0.4+0.8	0.15
5	1.0	0.1+0.2+0.4+0.8	1.5
6	10.0	0.1+0.2+0.4+0.8	15.0
7	100.00	0.1+0.2+0.3+0.4	100.00
8	100.00	0.5+0.6	110.00
Total cumulative dose			226.66665

Table 3. Characteristics of Boys With Grade III Systemic Reaction During Honeybee Rush VIT

	Patient 1	Patient 2	Patient 3
Dose which provoked SAR reaction during VIT, µg	30	20	3
Age, y	10	12	6
Pretreatment Mueller grade	III	III	III
Number of stings before reaction	10	2	0
Exposure to culprit insect	High	High	Medium
Atopy presence	No	No	Yes, AR, mild chronic BA
Total IgE, kU_A/L	262	489	80
Clinical symptoms of SAR/ provoking dose, µg	Urticaria, wheezing/ 3 µg	Urticaria, wheezing/ 20 µg	Sneezing, wheezing/ 30 µg
SSIgE to *Apis mellifera*, kU_A/L	6.3	101.0	10.3
SSIgE to *Vespula*, kU_A/L	0.64	1.92	0.34
SS *Apis mellifera* IgE/t IgE ratio	0.253	0.207	0.129
Baseline tryptase, µg/L	7.76	13.60	9.42
Tryptase after rush VIT	10.30	16.70	29.90
Baseline plasma 9α,11ß-PGF_2 concentration, pg/mL	3.80	6.70	2.40
Plasma 9α,11ß-PGF_2 concentration after rush VIT	11.80	24.90	9.70
Baseline urinary 9α,11ß-PGF_2 concentration, ng/mg creatinine	0.20	0.53	1.10
Urine 9α,11ß-PGF_2 concentration after rush VIT	0.20	0.59	0.40
Baseline urinary PGDM concentration	0.94	0.73	2.56
Urine PGDM after rush VIT, ng/mg creatinine	0.56	5.44	4.52
PEF, % normal values	110	98	89

Abbreviations: AR, allergic rhinitis; BA, bronchial asthma; Ig, immunoglobulin; PEF, peak expiratory flow; PG, prostaglandin; SAR, systemic adverse reaction; SSIgE, serum specific IgE; VIT, venom

The predictive value of PGD_2 metabolites for systemic adverse effects during VIT was estimated using a receiver operator characteristics (ROC) curve for each PGD_2 metabolite separately. The best sensitivity-to-specificity ratio was reported [17]. Positive or negative predictive value, defined as percentages of correctly classified cases with and without adverse systemic reactions, were computed. [18]. An area under the ROC curve (AUC) close to 0.5 means that prediction of a systemic adverse reaction using the predictor is no better than a result due to chance.

Results

Clinical Findings

Grade III systemic adverse events were observed in 3 boys, 1 of whom was atopic (Table 3). The results of baseline respiratory tests were normal, with no clinical symptoms of asthma in the pretreatment physical examination. The patients had not used ß-agonists within the 6 months before VIT. No systemic adverse events were observed during rush VIT in the other children.

Table 4. Comparison of Parameters Measured in 2 Detection Points for Children Allergic to *Vespula* Species and Honeybee

	Vespula Species		*Apis mellifera*	
Baseline serum tryptase concentration, μg/L	2.80 (1.11)	NS	5.06 (3.93)	*P*=.007
Serum tryptase concentration after rush VIT	2.72 (0.75)		8.34 (8.81)	
Baseline plasma 9α,11ß-PGF_2 concentration, pg/mL	3.99 (2.03)	NS	6. 55 (5.31)	*P*=.047
Plasma 9α,11ß-PGF_2 concentration after rush VIT	6.83 (6.76)		12.48 (8.96)	
Baseline urinary 9α,11ß-PGF_2 concentration, ng/mg creatinine	1.39 (1.72)	*P*=.021	0.56 (0.27)	NS
Urinary 9α,11ß-PGF_2 concentration after rush VIT	0.46 (0.33)		0.56 (0.24)	
Baseline tetranor-PGDM urinary concentration, ng/mg creatinine	1.28 (1.68)	NS	1.08 (0.68)	*P*=.028
Tetranor-PGDM urinary concentration after rush VIT	1.03 (0.78)		2.08 (1.78)	

Abbreviations: NS, nonsignificant; PG, prostaglandin; VIT, venom immunotherapy.

Baseline serum tryptase levels in children with no adverse reactions during rush VIT were significantly lower (*P*<.001) than in children with adverse reactions (no higher than 4.52 μg/L). No significant differences were observed in baseline tryptase values between children sensitized to *Vespula* species and children sensitized to *Apis mellifera* (Table 4).

Differences in the baseline tryptase level according to Mueller grade before VIT were significant. The lowest serum tryptase level was observed in children with a Mueller grade II reaction (1.93 [0.36] μg/L), while the highest was in children

Table 5. Arithmetic Means of Analyzed Parameters Before and After Rush VIT With Regard to Occurrence of an SAR

	Serum tryptase concentration μg/L		9α, 11ß-PGF_2 plasma concentration, pg/mL[c]		9α, 11ß-PGF_2 urine concentration, ng/mg creatinine		PGDM urine concentration, ng/mg creatinine[d]	
	Before	After	Before	After	Before	After	Before	After
Total	3.99	5.68	5.41	9.97	0.95	0.51	1.17	1.58
VIT and no SAR	2.81	3.19	5.63	8.87	1.01	0.53	1.13	1.22
VIT and SAR	10.26	18.97	4.30	15.47	0.61	0.40	1.41	3.51

Abbreviations: PG, prostaglandin; SAR, systemic adverse reaction; VIT, venom immunotherapy.
[a]Difference between children with and without SAR at baseline: *P*=.000
[b]Difference between children with and without SAR after rush VIT: *P*=.000
[c]Difference between total baseline and after VIT means: *P*=.032
[d]Difference between children with and without SAR after rush VIT: *P*=.009

with a Mueller grade III reaction (6.31 [4.80] μg/L; *P*=.029). Post hoc pairwise comparisons showed that the differences between median baseline tryptase level of grade II vs III and grade II vs IV were also significant (*P*=.048 and *P*=.014, respectively). After exclusion of the 3 children with grade III systemic adverse reactions during VIT, baseline tryptase concentration correlated positively with the Mueller grade (*P*=.024), although only the difference between grade II and grade IV retained its significance.

Baseline values of urinary 9α,11ß-PGF_2 were significantly lower in children with allergy to *Vespula* venom (3.99 [2.03] ng/mg of creatinine) than in children who were allergic to *Apis mellifera* venom (6.55 [5.31]; *P*=.023) (Table 4). After exclusion of the 3 children with grade III adverse reactions during rush VIT, this difference lost its significance.

We observed a negative correlation between age and baseline serum tryptase level (τ-b=–0.35; *P*=.044) and urinary 9α,11ß-PGF_2 excretion (τ-b=–0.37; *P*=.031).

A negative correlation between SSIgE and baseline urinary concentration of PGD_2 metabolites was observed. In children allergic to Vespula species, the correlation was negative between SSIgE and tetranor-PGDM (τ-b=–0.49; *P*=.006). Likewise, in children allergic to *Apis mellifera*, the correlation was negative between SSIgE and 9α,11ß-PGF_2 (τ-b=–0.38; *P*=.042).

Comparison of the Markers Measured Before and After Rush VIT

Gender and atopy did not affect changes in mediators at the 2 assessment points. Table 4 summarizes the comparisons of parameters between these samples separately for children allergic to *Vespula* species and children allergic to *Apis mellifera*. In the *Apis mellifera*–allergic group the markers increased following rush VIT, except for urine 9α,11ß-PGF_2. In children sensitized to *Vespula* species, the 9α,11ß-PGF_2 urine concentration was higher at baseline and lower following VIT. Mean serum tryptase and plasma 9α,11ß-PGF_2 were significantly higher in *Apis mellifera*–allergic children than in *Vespula*-allergic children; however, these differences disappeared when we excluded the 3 patients with systemic adverse reactions.

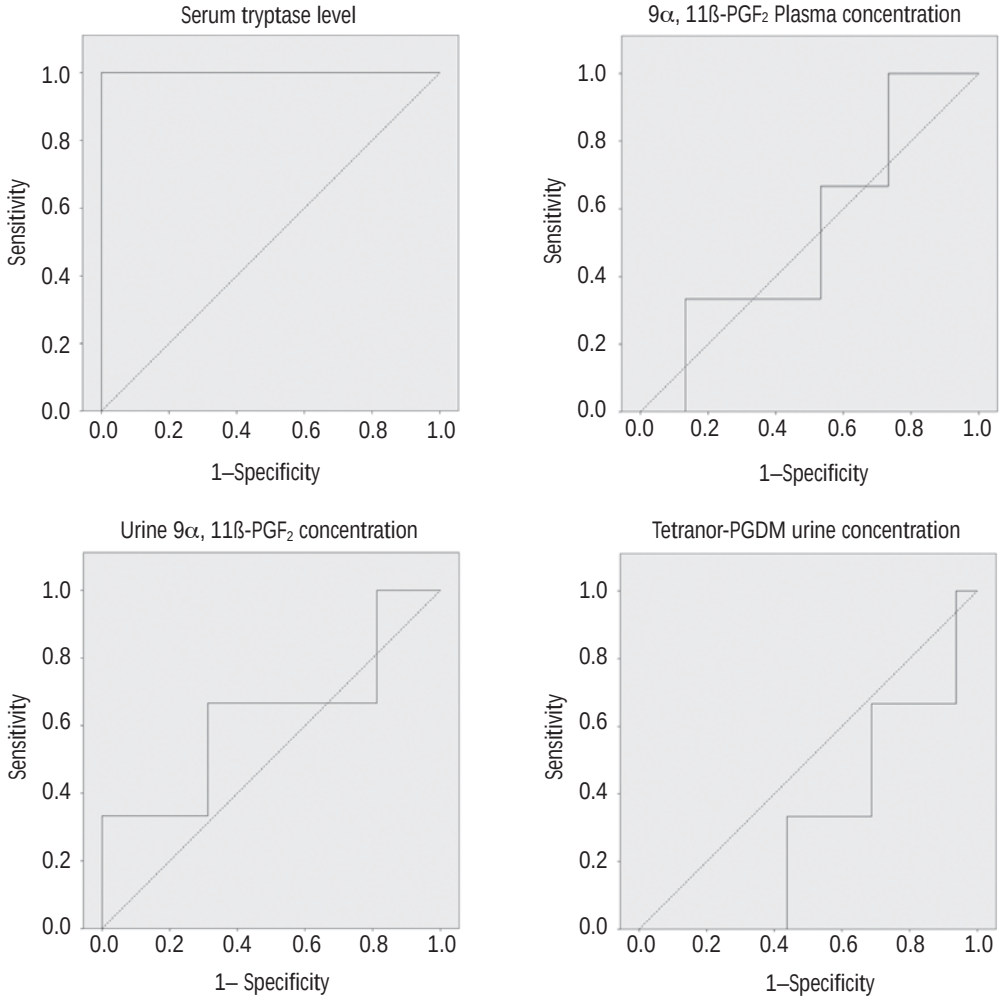

Figure 1. Receiver operating characteristic curves for parameters predicting the risk of severe systemic reaction to rush venom immunotherapy.

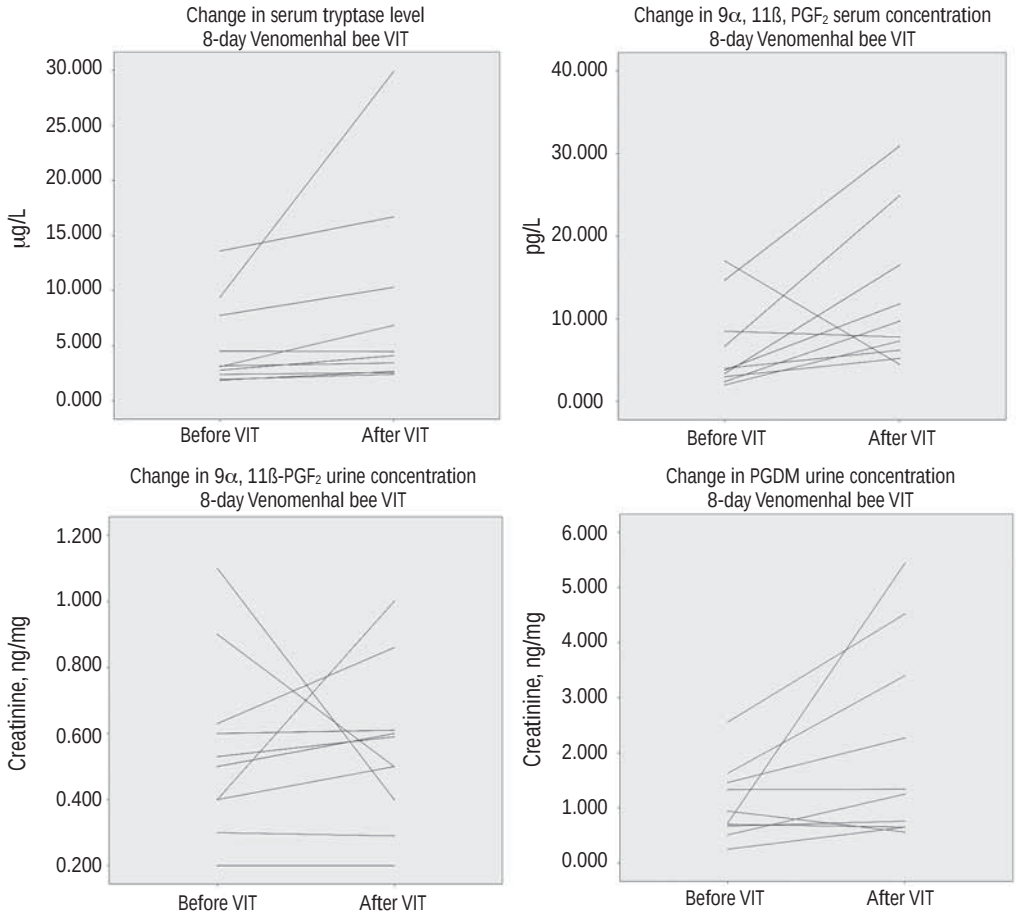

Figure 2. Individual excretion of serum tryptase, plasma 9α,11ß-PGF_2, urine 9α,11ß-PGF_2, and urine tetranor-PGD-M in children treated with specific immunotherapy to *Apis mellifera* venom. VIT indicates venom immunotherapy.

Children With Systemic Adverse Reactions During Buildup

Baseline serum tryptase levels in the children with systemic adverse reactions during VIT were higher than in children without reactions—the concentrations were higher than 7.76 µg/L in all 3. Table 5 summarizes the parameters analyzed before and after rush VIT and stratifies them according to the presence of a systemic adverse reaction. The markers in 2 of 3 patients with reactions were lower than the mean level in the group of children with no reactions during VIT. Serum tryptase increased almost 2-fold during VIT and urinary tetranor-PGD-M more than 2-fold. Plasma 9α,11ß-PGF_2 increased almost 5-fold in children with reactions, although this difference was not statistically significant (Table 5).

Impact of VIT

Repeated measures ANOVA showed that the factors with a significant impact on serum tryptase level were sampling points (ie, VIT treatment) (*P*=.001), occurrence of a systemic adverse reaction (*P*=.000), and the interaction between the two (*P*=.002). This parameter increased in both groups, but it was significantly greater in the children with an adverse reaction.

Analysis of plasma 9α,11ß-PGF_2 concentration showed only a significant impact for VIT (*P*=.011), which caused an increase in PG levels after immunization. No significant differences were observed between children who had an adverse reaction and those who did not.

The results of the analysis for urine tetranor-PGD-M revealed that both VIT (*P*=.006) and systemic adverse reaction (*P*=.019) had a significant impact on this metabolite. Immunization increased excretion of tetranor-PGD-M in urine in both groups, although the interaction between occurrence of a reaction and sampling point was of borderline significance (*P*=.052).

Urinary 9α,11ß-PGF_2 excretion was not significantly associated with VIT or adverse reactions.

Predicting the Properties of the Markers During Buildup

Serum tryptase proved to be an excellent predictor of systemic adverse reactions: all 3 children with anaphylactic symptoms during VIT had increased baseline levels (>7.76 µg/L).

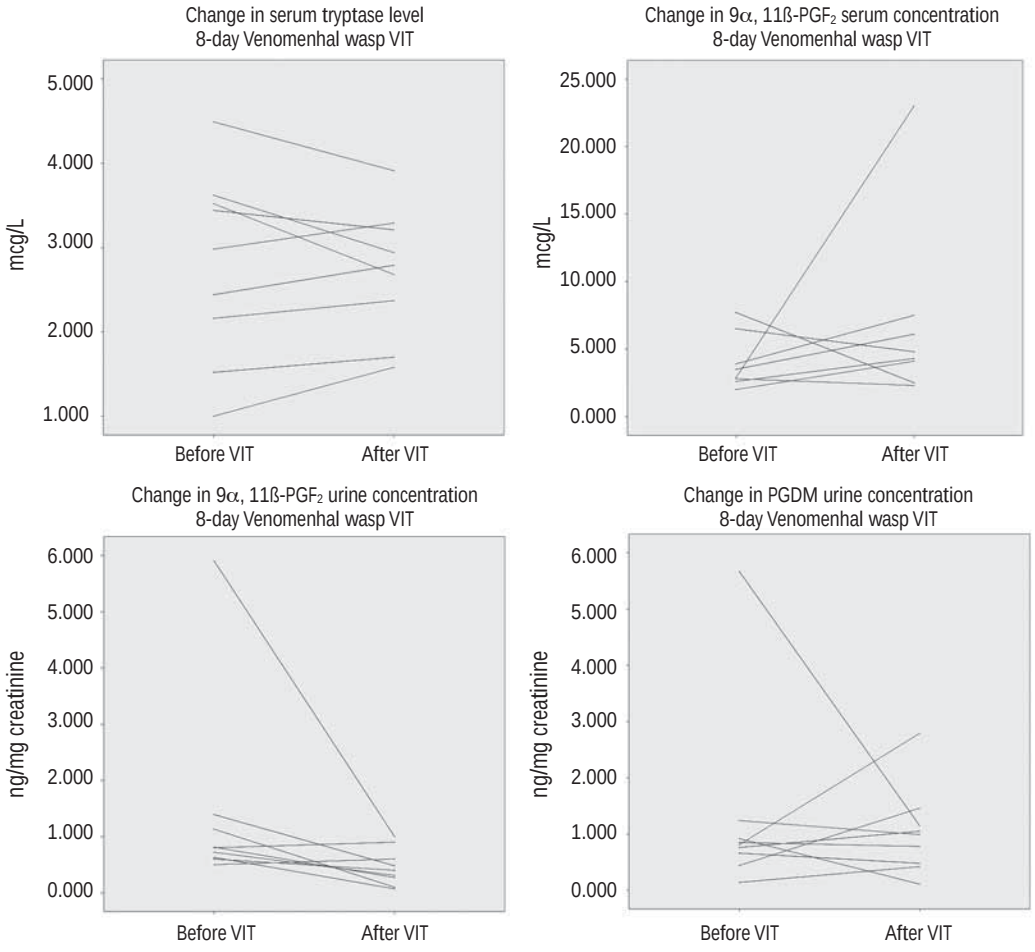

Figure 3. Individual excretion of serum tryptase, plasma 9α,11ß-PGF_2, urine 9α,11ß-PGF_2, and urine PGD-M concentration in children treated with specific immunotherapy to *Vespula* species. VIT indicates venom immunotherapy.

The other prostanoids were evaluated using ROC curves. The sensitivity of both markers was at best 67%, and specificity did not exceed 53%. The highest specificity was found for urine 9α,11ß-PGF_2 concentration, with a cutoff of 0.62 ng/mg of creatinine, assuming that lower values impose a higher risk of an adverse reaction. The area under the curve for 9α,11ß-PGF_2 was 60% (Figure 1), although this result was not significant. Neither of the prostanoid markers had a positive predictive value higher than 0.22; however, interestingly, the negative predictive value was not less than 83%. Hence, neither of the studied PGD metabolites was useful in predicting adverse reactions during VIT.

Individual levels of serum tryptase, plasma 9α,11ß-PGF_2, urinary 9α,11ß-PGF_2, and urinary tetranor-PGD-M concentrations in children treated with specific immunotherapy to *Apis mellifera* and *Vespula* species venom are presented in Figures 2 and 3.

Discussion

We analyzed a profile of specific mast cell–derived metabolites during rush VIT. To do so, we took into account the kind of venom sensitization (*Vespula* species and *Apis mellifera*) and systemic adverse effects during the buildup phase of the protocol. No published studies have evaluated serum tryptase in relation to plasma and urine concentrations of PGD_2 metabolites during rush VIT in children.

The main limitation of this study is its small population and male predominance. This may reflect a greater risk of exposure to stings by boys, who take part in more outdoor activities. More children were positive for venom SSIgE than for intradermal tests. In a few nonatopic children presenting high values of SSIgE, total IgE values were also elevated. Three children were atopic. The frequency of atopy in the study sample seemed comparable with that of the general population [1]. Three children had a systemic adverse reaction (Mueller grade III) to VIT, and 1 of these was diagnosed with atopic asthma. This finding is relevant, as uncontrolled asthma is the main risk factor of severe anaphylaxis in children [19]. We did not find any differences in the baseline values of mast cell mediators according to gender and atopy; this observation is consistent with published data [6,8]. The baseline laboratory parameter that allowed us to identify children with systemic

adverse reactions was significantly higher baseline serum tryptase levels. These children also had lowered urine 9α,11ß-PGF_2 values. Neither total IgE nor venom specific IgE were discriminative. VIT had an impact on the parameters analyzed in patients who had systemic reactions and in those who did not. These changes in the levels of mediators lost their significance after children with systemic adverse reactions to VIT were excluded. The change in urine 9α,11ß-PGF_2 values following VIT was puzzling and unexpected. Serum tryptase proved to be an infallible predictor of adverse reactions: all 3 children with baseline serum tryptase exceeding 7.76 μg/L had reactions. PGD_2 metabolites were poor predictors of systemic adverse reactions, and their positive predictive value was particularly unsatisfactory.

In children with no systemic adverse reactions, differences in levels of mast cell mediators did not change significantly following rush VIT. The treatment protocol seems safe, as it allowed the patients to tolerate a dose equal to several stings by *Vespula* species or more than 4 *Apis mellifera* stings within 8 days. All the patients with an adverse reaction during VIT had significantly higher baseline serum tryptase values, a finding that is consistent with those of other authors [20]. Ruëff et al [3,4] recently pointed out elevated baseline serum tryptase level as a predictor of severe systemic reaction both to field sting and during the buildup phase of VIT. In our study, the mean baseline tryptase level was 2.80 (1.11) μg/L for children allergic to *Vespula* species and 5.06 (3.93) μg/L in children allergic to *Apis mellifera*, while in children with systemic adverse reactions it exceeded 7.75 μg/L. Compared with data from a multicenter cohort study on predictors of anaphylactic reaction in adults [2], patients who did not have an adverse reaction in our study had baseline serum tryptase levels lower than the reference value 5.84 (8.36) μg/L. We conclude that a routine evaluation of mast cell mediators in children with severe systemic reaction to Hymenoptera stings might help in planning immunotherapy. The safety of rush VIT protocols in high-risk patients has already been described [21,22]. In high-risk patients, depot extracts should be considered during the maintenance phase [23]. It is important to investigate and control respiratory symptoms in asthmatic children before VIT. Our results are consistent with those of other authors, who recommend special monitoring of patients treated with bee venom [24].

The quality of allergen-specific immunotherapy should be monitored using national surveys [25]. Our model did not take into account circadian variation in serum tryptase level or the decline in serum tryptase level during long-term Hymenoptera VIT reported elsewhere [26,27].

The mass spectrometry technique applied to measure PGD_2 metabolites in our study has the highest specificity and sensitivity among the available methods used to analyze prostanoid compounds in biological matrices (plasma, urine, exhaled breath concentrate) [6-9,11,12,16]. Published data on monitoring PGD_2 metabolites in patients with bronchial asthma suggest this is a sensitive method for evaluation of PGD_2 biosynthesis following bronchial challenge in allergic asthma [6,8,9]. Only 1 paper reports urine 9α,11ß-PGF_2 levels as a more useful marker of anaphylaxis than serum tryptase in patients with a history of anaphylaxis that reoccurred during a provocation test to identify specific allergens [11]. The novel finding of our study was that the only laboratory parameters that allowed us to identify children at risk of systemic adverse reactions during VIT were higher baseline serum tryptase and lower urine 9α,11ß-PGF_2 levels. Even though VIT affected biomarker levels regardless of whether an adverse reaction occurred during VIT, these differences lost their significance after exclusion of children who experienced an adverse reaction. The finding of decreased urine 9α,11ß-PGF_2 levels following VIT merits further study, as it contrasts with the changes observed in other markers and could indicate common patterns of PGD_2 metabolism among children sensitized to Hymenoptera venom. However, reference concentrations of 9α,11ß-PGF_2 in plasma and urine are not currently available.

Our study provided conclusive evidence that rush VIT is safe in children with low baseline serum tryptase levels. Elevated serum tryptase level is a good predictor of severe systemic reactions during VIT.

Conclusions

Although clonal mast cell disorders are rare in children, evaluation of baseline serum tryptase levels should be a standard procedure to ensure optimal prognosis, monitoring, and administration of VIT following severe systemic reactions to *Hymenoptera* sting. Children sensitized to *Apis mellifera* venom and whose baseline serum tryptase exceeds 7.76 μg/L should be carefully monitored for systemic adverse reactions during the buildup phase of VIT. Likewise, in adults, there is a need for validation of cutoff values for baseline serum tryptase levels in much larger populations. This would help to identify children with a higher risk of systemic adverse reactions during VIT.

Lower plasma and urine 9α,11ß-PGF_2 concentrations are also associated with a higher risk of systemic adverse reactions during VIT. Since no reference values are available for this metabolite and interindividual variation is substantial, the hypothesis of underlying metabolic alterations should be tested using much larger populations of children.

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