Impact of Hymenoptera venom allergy and the effects of specific venom immunotherapy on mast cell metabolites in sensitized children*

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Abstract

Introduction and objective. Mast cells (MC) are effector cells during severe systemic reactions (SR) to Hymenoptera stings. Venom specific immunotherapy (VIT) is the treatment of choice for prevention of SR to stings. Tryptase and prostaglandin D_2 metabolites (PGD_2) are the markers of MC activation. The study design was to 1. compare baseline values of serum tryptase concentration (BST) and PGD_2 metabolites in children with/without venom sensitization, 2. to evaluate an influence of rush VIT on MC markers in treated children.
Materials and methods. Sensitized group: 25 children with SR to Hymenoptera sting. Control group: 19 healthy children. Active treatment: 5-day-rush-VIT. BST was evaluated by ImmunoCAP, PGD_2 metabolites in blood and urine by GC-NICI-MS.
Results. The baseline blood levels of MC markers were significantly higher, while urinary concentration of $9\alpha,11\beta$-PGF_2 was significantly lower in the whole group of venom-sensitized children compared to controls. Severity of SR showed negative correlation with urinary PGD_2 metabolites, while positive with plasma $9\alpha,11\beta$-PGF_2 and BST concentration The highest sensitivity was obtained for plasma $9\alpha,11\beta$-PGF_2 whereas the highest specificity for urinary PGD-M.
Conclusions. In children with IgE-mediated SR to *Hymenoptera* stings, elevation of baseline values of PGD_2 metabolites in blood is accompanied by decreased excretion of its urinary metabolites.
Assessment of stable PGD_2 metabolites might serve as an independent MC marker to identify allergic children.
There is an association between urinary PGD_2 metabolites and severity of the SR to *Hymenoptera* stings.

Key words

child, prostaglandin D_2 / secretion, tryptases / blood, bee venom / therapeutic use, wasp venom / therapeutic use, desensitization, immunologic/methods

INTRODUCTION

Venom allergy is an example of an acute hypersensitivity reaction, a type of systemic reaction that occurs in less than 1% of the children population, and which are usually less severe than in adults. In children with the history of life-threatening venom-triggered systemic IgE-mediated reactions, a 3- to 5-year course of subcutaneous specific venom immunotherapy (VIT) is a treatment of choice, even at age below 5 years, providing 98% rate of long-lasting protection against a subsequent sting [1,2]. Most studies concerning risk factors and mechanism of both venom allergy and immunoprotection following treatment have been conducted in adults [3]. It is not possible to extrapolate directly these results to children [4].

Systemic reactions (SR) due to *Hymenoptera* stings are mainly initiated by the IgE-mediated release of mediators from mast cells and basophils upon exposure to venom allergens. Despite the progress in diagnostic methods of IgE-mediated mechanisms of sting allergy, effector cells of anaphylaxis still remain the target of many studies in venom allergy [5].

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Demonstration of rapid, transient increase of serum tryptase level (enzymatically active mature β-tryptase) after specific allergen stimulation reflects massive mast cell activation and confirms the diagnosis of anaphylaxis [6] while baseline serum mast cell tryptase (BST) (inactive α,β-protryptases) concentration reflects the constitutive, individual mast cell load or activity. Values higher than 11.4 ng/mL are considered to be a marker of mast cell clonal disorders, including occult systemic mastocytosis [7]. Two recently published large cohort, multicenter studies allowed for the construction of a model/ identification of the characteristic risk factors of severe systemic reaction after *Hymenoptera* field stings as well as during the buildup phase of VIT in adults [8, 9]. BST elevated above 5.84 ng/mL is among the identified predictors. There are some data on increase of BST with age, which might explain aggravated sting allergic reactions in elderly people [10]. Prostaglandin D_2 is a major cyclooxygenase product released by activated mast cells. It is unstable and metabolized by NADPH-dependent 11-ketoreductase to $9\alpha,11\beta$-PGF_2, which is further transformed by oxidation to tetranor-PGD-M (PGD-M), both being rapidly excreted in urine [11, 12]. These early indicators of mast cell activation, were found to be useful in monitoring asthmatic adults [13, 14, 15] and children [16, 17]. Urinary concentration of $9\alpha,11\beta$-PGF_2 proved to be a reliable marker of endogenous production of inflammatory mediators associated with anaphylaxis [18].

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Objective. In our previous study we attempted to estimate the role of BST, as well as both blood and urinary prostaglandin D_2 metabolites, in predicting systemic side reaction during *Hymenoptera* venom immunotherapy in children [19]. In this study we wanted to compare the baseline values of prostaglandin D_2 metabolites and serum tryptase between symptomatic venom sensitized and healthy children, as well as to estimate the impact of rush schedule of venom immunotherapy on these parameters. There are no such studies in children.

MATERIALS AND METHOD

Study subjects. Sample of this prospective case-control study comprised two groups: a venom-sensitized group and a healthy control group. Venom-sensitized group included twenty five children (20 boys) aged 6–17 years (mean 11.5; SD 3.5;) fulfilling both inclusion criteria of 1. Systemic (grade II-IV according to Mueller's classification) reaction to *Hymenoptera* sting, children with the history of grade II of systemic reaction were qualified to VIT in case of increased exposure to stings (e.g. bee keepers families). 2. IgE-mediated venom allergy which was confirmed by positive result of at least one of the following procedures: a skin prick tests with venom extract concentration 100mcg/ml, intradermal tests with up-dosing to maximum concentration 1 mcg/ml, and specific serum IgE estimation by ImmunoCAP (Phadia AB, Uppsala, Sweden) were performed three to six weeks after systemic sting reaction. The venom extract used to skin testing as well as to treatment was obtained from the same manufacturer. Atopy was confirmed by positive skin prick test to inhalant allergens (*Allergopharma Joachim Ganzer KG*). After obtaining the results of diagnostic procedures venom-sensitized children were randomly divided into two groups of in-patient treatment: nineteen children (10 sensitized to *Apis m.* venom, 9 sensitized to *Vespula spp.* venom) to 5-day rush VIT with Venomenhal (HALAllergy, The Netherlands) (cumulative dose equal to 216.46 mcg), and 6 children (sensitized to *Apis m.* venom) to 5-day rush VIT with Pharmalgen (ALK Abello, Denmark) (cumulative dose equal to 223.3 mcg). The difference in cumulative dose was negligible. There is a common source (*Vespa Laboratories*, Penn, USA) of venom used by both manufacturers to produce the aqueous venom extract. The schedule of VIT was concordant with manufacturers' instruction. No antihistamines, systemic glucocorticosteroids, or LTRA were administered during VIT. On each VIT treatment day patients were examined for symptoms of respiratory tract infection, stable clinical status and PEF over 80% of normal value. Systemic side-reactions (SSR) during treatment were monitored.

Control group consisted of nineteen children enrolled from hospital out-patients' rehabilitation department, aged 7–11 years (mean age 9.1; SD 1.5; 8 boys) who fulfilled the following criteria: 1. Negative history of personal/family atopic diseases, no allergic sting reactions or autoimmunological disorders in phone administered interview with the parents of the child, 2. No atopic symptoms or *urticaria pigmentosa* on physical examination, 3. Negative skin prick tests with common inhalant allergens, milk and egg food allergens (*Allergopharma Joachim Ganzer KG*), negative serum specific IgE to *Apis m.* and *Vespula spp.* venoms

An informed consent to participate in the study was obtained from parents of the study subjects. The study protocol was approved by Jagiellonian University Ethical Committee.

COLLECTION OF SAMPLES

In the treated group, samples of fasting venous blood and morning urine were taken twice for estimation of the mast cell biomarkers: 1. at baseline, before first dose of rush VIT, 2. after the last injection of incremental dose – for $9\alpha,11\beta$-PGF_2 measurment blood was collected 5 minutes after injection of the venom extract, and one hour later for tryptase estimation. Urine sample was collected within 1–2 hours after the last injection. In the control group blood and urine samples were taken once to estimate basal serum tryptase level, plasma $9\alpha,11\beta$-PGF_2 and the first morning urine sample metabolite content. Time schedule of samples collection reflected metabolism of both mediators and was in line with other authors [12, 14].

Blood samples. for tryptase were allowed to clot, then centrifuged and serum stored at –80 °C. Fluoroenzyme immunoassay method using CAP System (Phadia, Uppsala, Sweden), which estimates both pro-forms and mature β tryptase, was performed. Serum tryptase detection ranges within 1–200 ng/mL, while values below are considered normal 10 ng/mL [20]. In case of values over 10 ng/mL we performed duplicate measurements. According to manufacturer, the inter-assay variability for tryptase levels is below 5% for the range between 1.0 and 100 ng/mL. Anticoagulated blood samples (citrate) for $9\alpha,11\beta$-PGF_2 were immediately centrifuged at 3500 rpm for 10 minutes and 500 pg deuterium labelled prostaglandin $F_{2\alpha}$ ($[^2H_4]$ $PGF_{2\alpha}$) as an internal deuterated standard (Cayman Chemicals, Ann Arbor, Mi, USA) was added to 1 ml plasma. To 0,2 ml of urine samples internal deuterated standards of prostaglandin $F_{2\alpha}$ ($[^2H_4]$ $PGF_{2\alpha}$) and PGD-M ($[^2H_6]$ tetranor-PGD-M) was also added. This compensated for analytes lost during sample preparations. All samples were stored at –80 °C and assayed within one month. Measurement of $9\alpha,11\beta$-PGF2 and PGD-M were performed using gas chromatography negative-ion chemical ionization mass spectrometry (GC-NICI-MS) (model 5896 series II; Hewlett Packard,Palo Alto,CA,USA) as described elsewhere [12, 13, 21]. The diagnostic ions were 569 *m/z* for $9\alpha,11\beta$-PGF_2 and 573 *m/z* for internal standard, while for PGD-M *m/z* 489 and *m/z* 495, respectively, where *m/z* denotes a mass-to-charge ratio: the quantity formed by dividing the mass of an ion by the unified atomic mass unit and by its charge number (positive absolute value)[22]. For plasma samples the detection limit was 1 pg/mL, while for urinary samples 0,5 ng/mg creatinine. The concentration for plasma samples was expressed in picogram per milliliter, for urinary samples in nanogram per milligram of creatinine.

Statistical analysis. Descriptive statistics were expressed as median (Me) and quartiles (Q1-Q3). Comparison of variables measured at two time points was done with Wilcoxon signed-rank test. For variables measured at the same time point comparison between two groups was done with Mann-Whitney test, while for more than two groups with Kruskal-Wallis test. Strength of relationship between variables was

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estimated with Kendall tau-b coefficient. General Linear Model (GLM) was used to adjust the influence of age on PGD-M level in relationship to other parameters. The predictive value of PGD_2 metabolites and BST for venom allergy was estimated using a receiver operator characteristics (ROC) curve for each PGD_2 metabolite and BST separately. The cut point for the best sensitivity-to-specificity ratio was reported [23]. An area under the ROC curve (AUC) which is not significantly different from 0.5 means that prediction of venom allergy using the marker is no better than a result due to chance. As the study was not of cross-sectional design, the positive (or negative) predictive values, defined as percentages of cases among positive results of test (or percentage of non-cases among negative results of test, respectively), were computed based on assumption that prevalence of venom allergy in general children population equals 0.5 (different authors report values ranging from 0.3 to 0.8) using formulas depending on sensitivity and specificity of the markers [24, 25, 26]. Differences were regarded statistically significant at 0.05 type I error level. Analysis were done using SPSS 15 for Windows.

RESULTS

Characteristics of the treated group. The patients' mean age was higher in *Apis m.*venom sensitized children. In *Vespula spp.* sensitized children no one had the history of anaphylactic shock after a sting. Four patients were atopic, two with clinical manifestation of controlled mild bronchial asthma, in two symptoms of perennial allergic rhinitis were present. Regarding standard diagnostic procedures total IgE level was significantly higher in venom allergic children in comparison to controls (p=0.001). In children sensitized to *Apis m.*, the absolute values of venom specific IgE were higher than values of specific IgE in those sensitized to *Vespula spp.* (Tab. 1).

Baseline prostaglandin D_2 metabolites and tryptase in children sensitized to *Hymenoptera* venom and control group. Gender and atopy did not influence the baseline level of mast cell biomarkers, while age showed significant negative correlation with urinary PGD-M at baseline (tau-b=-0.40, p=0.007).

In the whole group of children sensitized to *Hymenoptera* the baseline values of both mast cell markers in blood were significantly higher (p=0,025 for tryptase, and p<0,001 for 9α,11β-PGF_2, respectively), while baseline urinary concentration of 9α,11β-PGF_2 significantly lower (p<0,001) in comparison to the control group (Tab. 2).

Regarding the type of sensitization, the baseline urinary PGD-M concentration was the highest in the control group, while the lowest in *Apis m.*-sensitized group (p=0.011). The serum tryptase level did not differ in this comparison (Fig. 1). After adjustment for age, the difference in PGD-M urinary level between treated groups and controls was still significant (p=0.049). Both plasma and urinary concentration of 9α,11β-PGF_2 retained their significance of difference (p=0.001 and p=0.002, respectively) as well.

A comparison within venom allergic children revealed higher urinary PGD-M concentration in *Vespula* sensitized than in *Apis m.* sensitized children (p=0.02) (Tab. 2). After adjustment for age, the difference lost its significance (p=0,101).

For the whole group of venom allergic children Mueller's grade of systemic reaction after sting showed negative correlation with both urinary 9α,11β-PGF_2 and PGD-M concentrations at baseline. The median concentration of urinary PGD-M significantly decreased with the increase of Mueller's grade score (from 5.21 (3.94–10.23) ng/mg creatinine for the patients with grade II, to 2.12 (0.73–4.97) for the patients with grade III, and to 1.33 (0.43–2.86) for the patients with grade IV) (p=0.043). After adjustment for age, relationship between Mueller's grade score and PGD-M concentration was still significant (p=0.001). Regarding the type of sensitization, it was still significant in *Vespula* sensitized group (p=0.035), while it lost its significance in *Apis m.* group (p=0.087) (Fig 2). The median value of urinary 9α,11β-PGF_2 concentration was the highest (1,60 (1.10–3.19)) in patients with grade II reaction, the lowest (0.58 (0.34–1.10)) in patients with the history of grade III sting reaction, while intermediate (0.65 (0.57–2.39)) in patients with the history of anaphylactic shock (grade IV) (p=0.012). There was no correlation between Mueller's grade score and any mast cell mediators in plasma. A correlation coefficient between baseline both plasma and urinary 9α,11β-PGF_2 concentration was significantly negative (tau-b=-0.30, p=0.034) in the whole group of allergic children. Regarding the type of sensitization this correlation remained significant only in *Vespula* sensitized children (tau-b=-0.67, p=0.012) but not in *Apis m.* sensitized group (tau-b=-0.11, p=0.558). No correlation between mast cell metabolites was detectable in the control group.

Effects of venom immunotherapy on prostaglandin D_2 metabolites and serum tryptase in sensitized children. VIT caused a significant increase of serum tryptase, but not in PGD_2 metabolites in the whole treated group. Further analysis revealed, however, that increase of serum tryptase was significant for whole treated group (from 3.23 (2.41–5.06) to 3.63 (2.96–4.97) ng/mL; p=0.003) and *Apis m.* allergic children (from 3.34 (2.26–4.59) to 3.88 (2.99–4.83) ng/mL; p=0.010) (Fig.1).

Table 1. Characteristics of VIT treated and control groups

	Apis m. VIT group	*Vespid* VIT group	control group	Total
N	16	9	19	44
Gender: boys (%)	12 (75%)	8 (89%)	8 (42%)	28 (64%)
Age: mean ± SD	12.4±3.0	9.9±3.9	9±1.5	10.4±3.1
Mueller's classification grade				
II°	7 (44%)	4 (44%)		11 (44%)
III°	6 (38%)	5 (56%)		11 (44%)
IV°	3 (18%)	0		3 (12%)
Presence of atopy	3 (18%)	1 (11%)		4 (16%)
total IgE: Me (Q1-Q3) (kU/l)	137.5 (67.2–229.0)	91.8 (1.5–307.5)	16.4 (8.1–30.5)	
Vespid serum venom specific IgE: Me (Q1-Q3) (kU/l)	0.5 (0.0–1.1)	2.3 (1.1–18.1)	0.00	
Apis m. serum venom specific IgE: Me (Q1-Q3) (kU/l)	21.1 (8.5–46.9)	0.0 (0.0–0.4)	0.00	
Positive results of IDT	11 (69%)	5 (56%)	NP	
Positive results of SPT	0 (0%)	0 (0%)	NP	

IDT – intradermal tests, NP – not performed, SPT – skin prick tests, Me – median value, Q1 –25th percentile, Q3 –75th percentile

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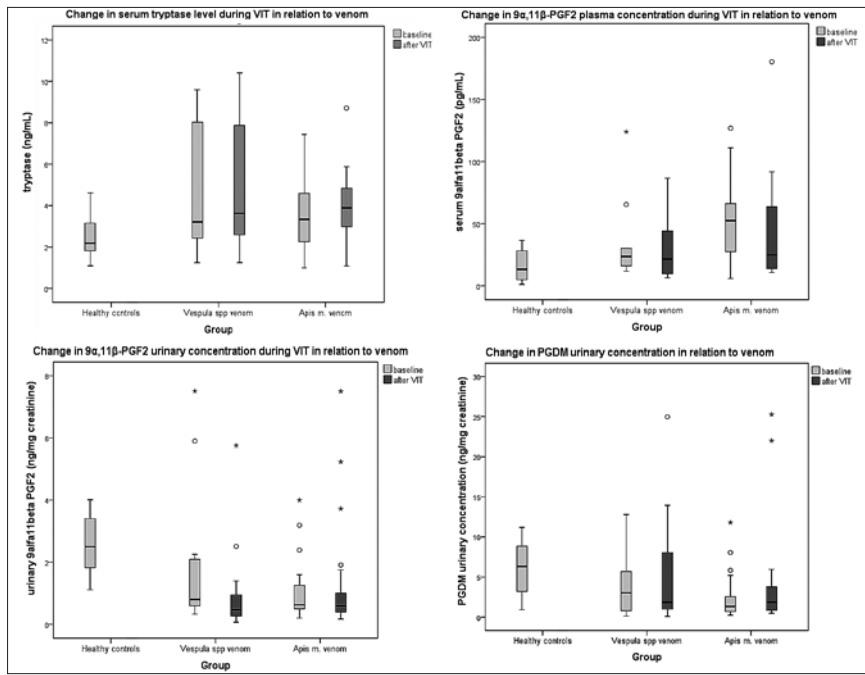

Figure 1. Change in mast cell markers concentration during induction phase of VIT with reference to type of venom sensitization, in comparison to healthy controls
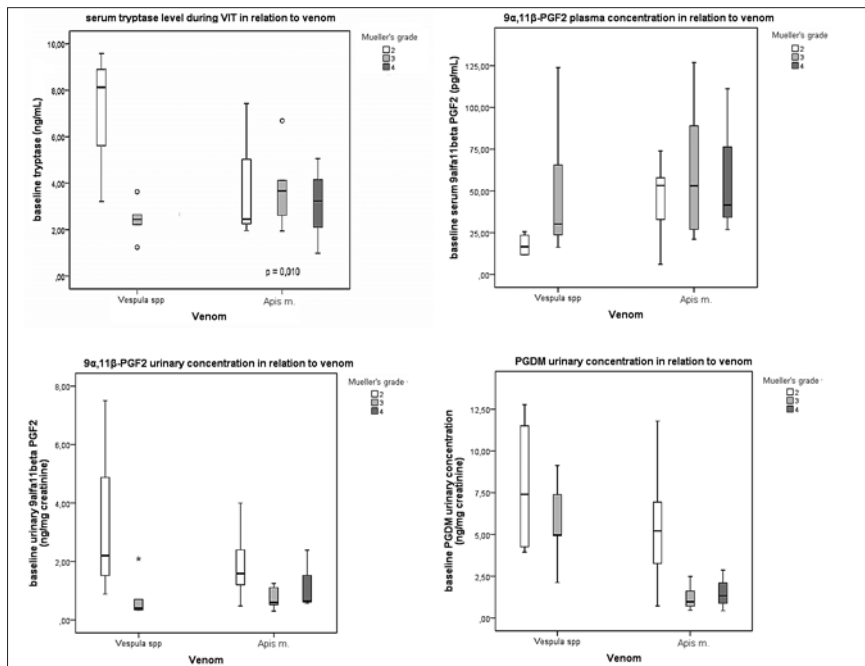

Figure 2. Relationship between Mueller's grade and mast cell markers concentration with reference to type of venom sensitization
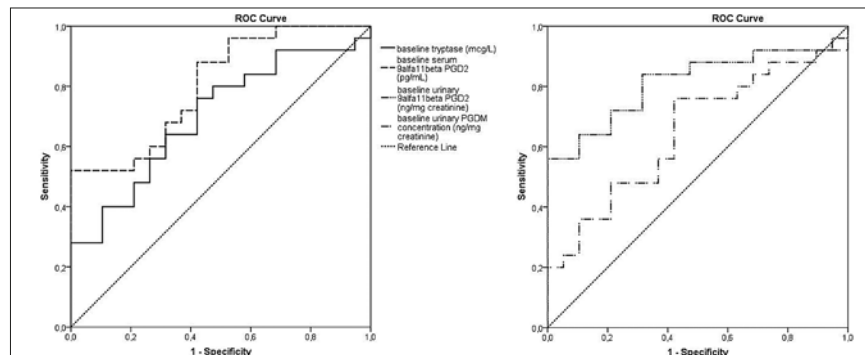

Figure 3. Receiver-operating (ROC) characteristics for blood and urinary mast cell markers

Table 2. Comparison of baseline median values (and interquartile ranges) of analyzed parameters between whole venom allergic group, regarding the type of venom sensitivity and control group

	serum tryptase (ng/mL)	plasma $9\alpha,11\beta$-PGF_2 (pg/ mL)	urinary $9\alpha,11\beta$-PGF_2 (ng/mg creatinine)	urinary PGD-M (ng/mg creatinine)
Vespula spp. allergic group	3.21 (2.43–8.03)	23.72 (14.03–47.85)	0.88 (0.37–2.20)	4.97 (4.26–9.68)
Apis melifera allergic group	3.34 (2.26–4.59)	52.52 (27.28–70.05)	1.10 (0.57–1.60)	1.54 (0.72–5.17)
whole group of venom allergic children	3.23 (2.41–5.06)	37.70 (22.52–62.00)	1.10 (0.54–2.12)	3.94 (1.26–6.61)
Control group	2.18 (1.75–3.29)	13.32 (4.70–28.60)	2.50 (1.74–3.47)	6.31 (3.08–9.26)
Comparison between whole venom allergic group and control group	p=0.025	p=0.001	p< 0.001	p=0.095
Comparison between *Vespula spp* and *Apis melifera* venom allergic children and controls	p=0.077	p=0.001	p= 0.002	p=0.011
Comparison between *Vespula spp* and *Apis melifera* venom allergic children groups	p=0.637	p=0.084	p=1.000	p=0.020

The relative increase of serum tryptase concentration in reference to the baseline value (relative delta bound) ranged from 88% to 158%. In four patients a relative increase ≥ 135% of the BST was observed, in one patient it was close to this bound. In spite that fact, immunotherapy was well tolerated, none of treated patients demonstrated systemic side effects during 5-day rush VIT.

There was no significant difference between baseline and post treatmentVIT values in PGD_2 metabolite concentration. However, regardless the type of venom sensitization both plasma and urinary median concentration decreased, while PGD-M median concentration increased during induction phase treatment (Fig. 1).

Serum tryptase at baseline, correlated positively with its level after treatment in whole treated group (tau-b=0.79, p<0.001), and in subgroups analyzed for the type of sensitization (tau-b=0.78, p=0.004 and tau-b=0.83, p<0.001 in *Vespula spp* and *Apis m.* sensitized group, respectively). Urinary $9\alpha,11\beta$-PGF_2 concentration after treatment correlated positively with its basal level only in *Apis m.* venom treated children (tau-b=0.48, p=0.010), while PGD-M pre/after VIT concentration correlated negatively in *Vespula spp* treated children (tau-b=–0.56, p=0.037).

Predictive properties of the markers to distinguish venom allergic children from controls. Baseline concentrations of both blood markers and urine $9\alpha,11\beta$-PGF_2 evaluated using ROC curve, revealed the potency to discriminate between venom allergic children and controls (Figure 3). The area under the curve for those three markers reached at least 0.7, being significantly different from value of 0.5, what reflects probability of random assignment of patient as allergic. The highest value of AUC and the optimal sensitivity/specifity ratio (for cut point of 1,67 ng/mg creatinine) in case of urinary $9\alpha,11\beta$-PGF_2 was achieved. Positive predictive values estimated for analyzed markers were all lower than 1.5% reaching 1.0% for tryptase and plasma $9\alpha,11\beta$-PGF_2. Negative predictive values were estimated as higher than 99.6% reaching 99.9% for both plasma and urine $9\alpha,11\beta$-PGF_2 (Tab. 3).

DISCUSSION

The reasons why some cases develop allergic reactions from bee and wasp stings, while others do not, still remains a mystery. An answers to this question is being searched for from among a variety of possible influences, such as environmental

Table 3. Utility of the mast cell markers in indicating venom hypersensitivity

	Baseline serum tryptase	Plasma $9\alpha,11\beta$-PGF_2	Urinary $9\alpha,11\beta$-PGF_2	Urinary PGD-M
AUC	0.70	0.80	0.81	0.65
p for difference between AUC and 0.5	0.025	0.001	<0.001	0.095
Sensitivity	0.64	0.88	0.79	0.58
Specificity	0.68	0.58	0.72	0.76
Cut point Unit of measurement	2.57 ng/mL	15.57 pg/mL	1.67 ng/mg creatinine	6.06 ng/mg creatinine
Positive predictive value	1.0	1.0	1.4	1.2
Negative predictive value	99.7	99.9	99.9	99.7

factors on allergy, as well as in more sophisticated fields, like genetic aspects of venom allergy or evaluation of effector cells (mast cells and basophils) markers [27, 28, 29]. Our case-control study represents the first – and novel – attempt to compare the baseline values of prostaglandin D_2 metabolites and serum tryptase between symptomatic venom sensitized and healthy children. We investigated also how 5-day venom specific rush VIT influences these parameters.

All *Hymenoptera* venom sensitized children were diagnosed in line with EAACI and AAAAI guidelines [1, 30, 31]. To achieve reliable laboratory results all drugs which may interfere with clinical symptoms (NSAID, LTRAs, antihistamines and systemic glucocorticosteroids) were withdrawn [32].

We did not find differences in level of mast cell markers between genders in venom allergic children, what might be due to the predominance of boys in the study group, which was also reported by other authors [33, 34]. In our study, the age of children showed a negative correlation with urinary PGD-M levels, but not with the other mast cell mediators, which is similar to the results obtained by Kielbasa et al. in their study on eicosanoids measurements in exhaled breath condensate of asthmatic children after provocation test [16]. Considering the type of venom sensitization and adjustment for the age of subjects, especially $9\alpha,11\beta$-PGF_2 concentration in both biological fluids behaved more stable than urinary PGD-M or serum tryptase concentration, thus indicating that $9\alpha,11\beta$-PGF_2, a PGD_2 metabolite, might provide better discriminating of venom allergic children from controls.

In venom sensitized children none of mast cell mediators measured in blood at baseline had discriminating power

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to distinguish between the type of sensitizing venom or severity of past anaphylactic reaction. An observation of the lower blood concentration of mast cell mediators in venom allergic children, as well as, the higher concentration in urine in control group, is consistent with the measurements results in which both urinary PGD_2 metabolites revealed the negative association with Mueller's grade in case of whole group of venom allergic children, as well as separately, in *Vespula spp.* allergic children. Hence, indicating that higher baseline values of urinary PGD_2 metabolites can discriminate not only sensitized children from healthy ones, but also might be useful in indicating those with milder systemic reaction. It is in line with the results of our previous study, in which we described the higher urinary $9\alpha,11\beta$-PGF_2 concentration in children without severe SR to VIT in comparison to patients with severe SR [19]. We speculate that higher urinary excretion may reflect some protective mechanisms. The similar phenomenon – of higher PGD_2 metabolites values in milder symptoms – was observed by Bochenek et al. in asthmatic patients stratified by severity of disease [14]. It is tempting to speculate that in severe systemic reactions to *Hymenoptera* stings, catabolism of PGD_2 to its inactive metabolites does not accelerate in response to its higher systemic biosynthesis.

We observed significant difference in baseline values of serum tryptase between venom allergic children and controls (3.23 ng/mL vs. 2.18 ng/mL). The similar data were reported by Yavuz in the age and sex-matched sample of Turkish children with insect venom allergy, showing that median level of BST in children with insect sting systemic reaction was significantly higher than in children with large local reaction and healthy subjects (4.2 vs 3.1 vs. 2.9 ng/mL, respectively) [35]. On the other hand in Komarow's paper on 197 children (age range: 6 months to 18 years) there was no statistically significant difference between non-atopic and atopic subjects (3.44 vs 3.56 ng/mL); though venom allergy was not included into study group [36].

During 5-day rush venom specific immunotherapy with aqueous venom extract none of patients demonstrated systemic side effects (non-reactors). In our study, the median value of BST were equal to 3.21 ng/mL for *Vespula spp.* allergic group and to 3.34 ng/mL for *Apis m* allergic group, which are below the median value 4.25 ng/mL from the cohort multicenter study, in which odds ratio of severe systemic reaction after sting in adults increased markedly when BST concentration was higher than 5 ng/mL [8]. In our recent study, we noted that children sensitized to *Apis m.* venom with mean value of serum tryptase level exceeding 7.75 ng/mL were at risk for systemic reaction to venom injection during rush protocol [19]. Other authors also reported that total tryptase level could indicate a risk for systemic reactions to rush immunotherapy and mast cell activation [37, 38]. Estimating the response to VIT, in comparison pre/post rush VIT values of mast cell mediators, only serum tryptase level, increased significantly, though within normal range, in the whole treated group and in *Apis m.* allergic subgroup. This is in line with the results pointing out that especially incremental phase of immunotherapy with bee venom extract goes together with the higher risk of systemic side effects and so this phase of treatment requires special attention [39]. Some authors pointed to an association between hypersensitivity reaction in adult venom allergic patients and a relative increase of serum tryptase level to $\geq 135\%$

of the baseline value [40]. In our study, only four of twenty five patients had this parameter exceed that limit. As none of our patients had reaction to venom extract, data from more patients will be necessary to validate this finding. The schedule of samples collection in our study did not allow us to estimate the circadian rhythm of mast cells mediators concentration in children [41].

In contrast to BST compared during pre/post rush VIT, PGD_2 metabolites concentrations were stable, with non-significant decrease of $9\alpha,11\beta$-PGF_2 both plasma and urinary concentration and non-significant increase of PGD-M, which may reflect observed safety of both rush-VIT protocol and venom extract used in treatment. In addition to the abundant PGD-M, a tetranor end product of oxidation, used in our study [42], a new eicosanoid-derived urinary biomarker 2,3-dinor-$9\alpha,11\beta$-PGF_2 emerged as the useful for monitoring symptomatic anaphylaxis and aspirin–intolerant asthma [43, 44]. Authors suggested that it might be the most reliable indicator in determining PGD_2 production *in vivo*, thus indicating that PGD_2 metabolites are mediators of special interest in studies dedicated to allergy.

At baseline, the plasma levels of PGD_2 metabolite were elevated, while urinary PGD_2 metabolites were decreased, discriminating venom allergic children from control group. Analysis of ROC curve properties showed acceptable parameters for all markers except urinary PGD-M, for which areas under curve were significantly higher that value of 0.5, reflecting probability of random assignment of the patient as venom-allergic or not. Among these three parameters, however, only for plasma $9\alpha,11\beta$-PGF_2 the ROC curve does not intersects the reference line, indicating it as even better marker of venom hypersensitivity than BST. This suggests that for a very low level of tryptase and for a very high level of urinary $9\alpha,11\beta$-PGF_2, these markers may wrongly classify healthy children as venom allergic ones, and vice versa. This may be due to the fact that control group in this study was smaller in size than the allergic one, what may underrepresent number of healthy children with the lowest values of BST. This might lead to situation that the smallest level of tryptase were observed in allergic children, although when comparing allergic and control group, the second one had significantly lower level of BST. Extended number of subjects in future studies are necessary to validate here reported observations. Though mast cells are the main effector cells of anaphylaxis in comparison to basophils, an attempts to evaluate venom allergy with basophil markers, are much more advanced in comparison to PGD_2 metabolites, originating exclusively from mast cells. Basophil activation test (BAT) has been introduced in both diagnosis and VIT monitoring of venom allergic patients [45, 46, 47, 48]. There are also attempts to introduce it as an useful diagnostic tool in IgE-negative patients with the history of systemic sting reaction [49]. The first, very interesting data on blood basophils as a target for VIT, indicated for impaired release of both preformed and newly generated mediators during 3,5-hours lasting ultra-rush VIT protocol [50]. Just recently preliminary data on simultaneous evaluation of BAT and mast cell markers during first 40 days of VIT introduction (build-up phase and two maintenance doses) was published recently. Authors concluded that plasma levels of $9\alpha,11\beta$-PGF2 decrease while numbers of activated basophils increase during the initial phase of bee venom rush immunotherapy in children [51].

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CONCLUSIONS

Children with history of IgE-mediated systemic reaction to *Hymenoptera* stings are characterized by elevated baseline values of mast cell metabolites in blood, and by decreased urinary PGD_2 stable metabolite concentration, that discriminates them from non-allergic children. Thus, assessment of the stable urinary PGD_2 metabolite might serve as an independent marker of mast cell activation in these patients. There is a negative association between urinary PGD_2 metabolites and severity of the systemic reactions. Uneventful rush venom therapy is not associated with any changes in either plasma or urinary levels of PGD_2 stable metabolites.

REFERENCES

1. Bilo BM, Rueff F, Mosbech H, Bonifazi F, Oude-Elberink JN. Diagnosis of Hymenoptera venom allergy. Allergy 2005; 60(11): 1339–1349.
2. Brown TC, Tankersley MS. The sting of the honeybee: an allergic perspective. Ann Allergy Asthma Immunol. 2011; 107(6): 463–470.
3. Shin YS, Liu JN, Hur GY, Hwang EK, Nam YH, Jin HJ, et al. Clinical Features and the Diagnostic Value of Component Allergen-Specific IgE in Hymenoptera Venom Allergy. Allergy Asthma Immunol Res. 2012; 4(5): 284–289.
4. Golden DBK. Insect sting allergy and venom immunotherapy. Ann Allergy Asthma Immunol. 2006; 96(2 Suppl 1): 16–21.
5. Akkoc T, Akdis M, Akdis CA. Update in the mechanisms of allergen-specific immunotherapy. Allergy Asthma Immunol Res. 2011; 3(1): 11–20.
6. Simons FER. World Allergy Organization survey on global availability of essentials for the assessment and management of anaphylaxis by allergy immunology specialists in health care settings Ann Allergy Asthma Immunol. 2010; 104(5): 405–412.
7. 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(3): 680–686.
8. Rueff F, Przybilla B, Biló MB, Müller U, Scheipl F, Aberer W, et al. Predictors of severe systemic anaphylactic reactions to 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(5): 1047–1054.
9. Rueff F, Przybilla B, Biló MB, Müller 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(1): 105–111.
10. Guenova E, Volz T, Eichner M, Hoetzenecker W, Caroli U, Griesinger G, et al. Basal serum tryptase as risk assessment for severe Hymenoptera sting reactions in elderly. Allergy. 2010; 65(7): 919–923.
11. O'Sullivan S, Mueller MU, Dahlen SE, Kumlin M. Analyses of prostaglandin D2 metabolites in urine: comparison between enzyme assay and negative ion chemical ionization gas chromatography mass spectrometry. Prostaglandins Other Lipid Mediat. 1997; 57(2–3): 149–165.
12. Tsikas D. Application of gas chromatography mass spectrometry and gas chromatography tandem mass spectrometry to assess in vivo synthesis of prostaglandins, thromboxane, leukotrienes, isoprostanes and related compounds in humans. J Chromatogr B Biomed Sci Appl. 1998; 717(1–2): 201–245.
13. Bochenek G, Niżankowska E, Gielicz A, Świerczyńska M, Szczeklik A. Plasma 9alpha,11beta-PGF2, a PGD2 metabolite, as a sensitive marker of mast cell activation by allergen in bronchial asthma. Thorax 2004; 59(6): 459–464.
14. Bochenek G, Nagraba K, Nizankowska E, Szczeklik A. A controlled study of 9alpha,11beta-PGF2 (a prostaglandin D2 metabolite) in plasma and urine of patients with bronchial asthma and healthy controls after aspirin challenge. J Allergy Clin Immunol. 2003; 111(4): 743–749.
15. Misso NLA, Aggarwal S, Phelps S, Beard R, Thompson PJ. Urinary leukotriene E4 and 9α,11β-PGF2 concentrations in mild, moderate and severe asthma, and in healthy subjects. Clin Exp Allergy 2004; 34(4): 624–631.
16. Kiełbasa B, Moeller A, Sanak M, Hamacher J, Hutterli M, Cmiel A, et al. Eicosanoids in exhaled breath condensates in the assessment of childhood asthma. Pediatr Allergy Immunol. 2008; 19(7): 660–669.
17. Nagakura T, Obata T, Shichijo K, Matsuda S, Sigimoto H, Yamashita K, et al. GC/MS analysis of urinary excretion of 9alpha,11beta-PGF2 in acute and exerice-induced asthma in children. Clin Exp Allergy. 1998; 28(2): 181–186.
18. Ono E, Taniguchi M, Mita H, Fukutomi Y, Higashi N, Miyazaki E, et al. Increased production of cysteiny leukotriens and prostaglandin D2 during human anaphylaxis. Clin Exp Allergy. 2008; 39(1): 72–80.
19. Cichocka-Jarosz E, Sanak M, Szczeklik A, Brzyski P, Gielicz A, Pietrzyk JJ. Serum tryptase level is better predictor of systemic side effects during venom specific immunotherapy in children than PGD2 metabolites. J Investig Allergol Clin Immunol. 2011; 21(4): 260–269.
20. Schwartz LB. Diagnostic value of tryptase in anaphylaxis and mastocytosis. Immunol Allergy Clin North Am. 2006; 26(3): 451–463.
21. Sanak M, Gielicz A, Nagraba K, Kaszuba M, Kumik J, Szczeklik A. Targeted eicosanoids lipidomics of exhaled breath condensate in healthy subjects. J Chromatogr B. 2010; 878(21): 1796–1800.
22. Brenton AG, Godfrey AR. Accurate mass measurement: terminology and treatment of data. J Am Soc Mass Spectrom. 2010; 21(11): 1821–1835.
23. Zweig MH, Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. Clinical Chemistry 1993; 39(4): 561–577.
24. Zou KH, O'Malley AJ, Mauri L. Receiver-operating characteristic analysis for evaluating diagnostic tests and predictive models. Circulation 2007; 115(5): 654–657.
25. Bilò BM, Bonifazi F. Epidemiology of insect-venom anaphylaxis. Curr Opin Allergy Clin Immunol. 2008; 8(4): 330–337.
26. Altman, DG; Bland, JM. Diagnostic tests 2: Predictive values. BMJ. 1994; 309(6947): 102.
27. Jenerowicz D, Silny W, Dańczak-Pazdrowska A, Polańska A, Osmola-Mańkowska A, Olek-Hrab K. Environmental factors and allergic diseases. Ann Agric Environ Med. 2012; 19(3): 475–481.
28. Karakis GP, Sin BA, Tutkak H, Kose K, Misirligil Z. Genetic aspects of venom allergy: association with HLA class I and class II antigenes. Ann Agric Environ Med. 2010; 17(1): 119–123.
29. McGovan EC, Saini S. Update on the performance and application of basophil activation tests. Curr Allergy Asthma Rep 2013;13(1):101–109.
30. Cox L, Williams B, Sicherer S. Pearls and pitfalls of allergy diagnostic testing: report from the American College of Allergy, Asthma and Immunology/American Academy of Allergy, Asthma and Immunology Specific IgE Test Task Force. Ann Allergy Asthma Immunol. 2008; 101(6): 580–592.
31. Bernstein IL, Li JT, Bernstein DI, Hamilton R, Spector SL, Tan R, et al. Allergy diagnostic testing: an updated practice parameter. Ann Allergy Asthma Immunol. 2008; 100(3 suppl 3): 1–148.
32. Breslow RG, Caiado J, Castells MC. Acetylsalicylic acid and montelukast block mast cell mediator–related symptoms during rapid desensitization Ann Allergy Asthma Immunol. 2009; 102(2): 155–160.
33. Jenning A Duggan E, Perry IJ, Hourihane JO. Epidemiology of allergic reactions to hymenoptera stings in Irish school children. Pediatr Allergy Immunol. 2010; 21(8): 1166–1170.
34. Martinez-Canavate A, Tabar AI, Eseverri JL, Martín F, Pedemonte-Marco C. An epidemiological survey of hymenoptera venom allergy in the Spanish paediatric population. Allergol Immunopathol (Madr). 2010; 38(5): 259–262.
35. Yavuz ST, Sackesen C, Sahiner UM, Buyuktiryaki B, Arik Yilmaz E, Sekerel BE, Soyer OU, Tuncer A. Importance of serum basal tryptase levels in children with insect venom allergy. Allergy 2013;68(3): 386–391.
36. 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(4): 845–848.
37. Vegh AB, George KC, Lotfi-Emran S. Total tryptase levels indicate risk for systemic reactions to rush immunotherapy and mast cell activation. Ann Allergy Asthma Immunol. 2011; 106(4): 342–343.
38. Kucharewicz I, Bodzenta-Lukaszyk A, Szymanski W, Mroczko B, Szmitkowski M. Basal serum tryptase level correlates with severity of hymenoptera sting and age. J Investig Allergol Clin Immunol. 2007; 17(2): 65–69.
39. Gorska L, Chelminska M, Kuziemski K, Skrzypski M, Niedoszytko M, Damps-Konstanska I, et al. Analysis of safety, risk factors and pretreatment methods during rush Hymenoptera venom immunotherapy. Int Arch Allergy Immunol. 2008; 147(3): 241–245.
40. Borer-Reinholdt M, Haeberli G, Bitzenhofer M, Jandus P, Hausmann O, Fricker M, et al. An increase in serum tryptase even below 11,4 ng/ml may

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indicate a mast cell mediated hypersensitivity reaction: a prospective study in Hymenoptera venom allergic patients. Clin Exp Allergy. 2011; 4(12): 1777–1783.

41. Dugas-Breit S, Przybilla B, Schopf P, Rueff F. Possible circadian variation of serum mast cell tryptase concentration. Allergy. 2005; 60(5): 689–692.

42. Song WL, Wang M, Ricciotti E, Fries S, Yu Y, Grosser T, et al. Tetranor PGDM, an abundant urinary metabolite reflects biosynthesis of prostaglandin D2 in mice and humans. J Biol Chem. 2008; 283(2): 1179–1188.

43. Higashi N, Mita H, Ono E, Fukutomi Y, Yamaguchi H, Kajiwara K, et al. Profile of eicosanoid generation in aspirin-intolerant asthma and anaphylaxis assessed by new biomarkers. J Allergy Clin Immunol. 2010; 125(5): 1084–1091.

44. Higashi N, Mita H, Yamaguchi H, Fukutomi Y, Akiyama K, Taniguchi M. Urinary tetranor-PGDM concentrations in aspirin-intolerant asthma and anaphylaxis. J Allergy Clin Immunol. 2012; 129(2): 557–559.

45. Ebo DG, Bridts CH, Hagendorens MM, De Clerck LS, Stevens WJ. The basophil activation test in the diagnosis and follow-up of hymenoptera venom allergy: an alternative point of view. J Investig Allergol Clin Immunol 2008; 18(6): 493–494.

46. Celesnik N, Vesel T, Rijavec M, Silar M, Erzen R, Kosnik M, Kloft Zitnik SE, Avcin T, Korosec P. Short-term venom immunotherapy induces desensitization of FcepsilonRI-mediated basophil response. Allergy 2012; 67(12): 1594–1600.

47. Nullens S, Sabato V, Faber M, Leysen J, Bridts CH, De Clerck LS, Falcone FH, Maurer M, Ebo DG. Basophilic histamine content and release during venom immunotherapy: Insights by flow cytometry. Cytometry B Clin Cytom 2013; 84(3): 173–178.

48. Zitnik SE, Vesel T, Avcin T, Silar M, Kosnik M, Korosec P. Monitoring honeybee venom immunotherapy in children with the basophil activation test. Pediatr Allergy Immunol 2012; 23(2): 166–172.

49. Korosec P, Erzen R, Silar M, Bajrovic N, Kopac P, Kosnik M. Basophil responsiveness in patients with insect sting allergies and negative venom-specific immunoglobulin E and skin prick test results. Clin Exp Allergy. 2009; 39(11): 1730–1737.

50. Jutel M, Muller UR, Fricker M, Rihs S, Pichler WJ, Dahinden C. Influence of bee venom immunotherapy on degranulation and leukotriene generation in human blood basophils. Clin Exp Allergy. 1996; 26(10): 1112–1118.

51. Cichocka-Jarosz E, Dorynska A, Pietrzyk JJ, Spiewak R. Laboratory markers of mast cell and basophil activation in monitoring rush immunotherapy in bee venom-allergic children. Immunotherapy 2011; 3(8): 1013–1017.