

Baseline Serum Tryptase Levels and Adverse Reactions to Injection Specific Immunotherapy with Airborne Allergens: Is There a Relationship?

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Key Words

Tryptase • Mast cells • Respiratory allergy • Immunotherapy

Abstract

Background: Elevated baseline serum tryptase levels are associated with severe systemic reactions following hymenoptera stings or venom immunotherapy. Little is known about baseline tryptase levels in patients with respiratory allergy and whether a relationship exists with systemic reactions induced by injection specific immunotherapy (SIT) with airborne allergens. The objective of this study was to measure tryptase levels in subjects with respiratory allergy and analyze the results in the light of tolerance/intolerance to injection SIT. **Methods:** Baseline serum tryptase levels were measured in 106 adults allergic to different airborne allergens and in 40 normal controls. Thirty-one patients underwent injection SIT, and 15 of these 31 experienced at least one SIT-induced systemic reaction. **Results:** Patients and normal controls showed similar median tryptase levels (2.98 vs. 3.13 ng/ml, respectively), although these were elevated in 6 patients (6%) versus 0 of 40 controls (0%). Tryptase levels did not differ between those patients with or without a history of systemic reactions (median 3.7 vs. 5.91 ng/ml, not significant). Three of 4 patients showing elevated tryptase levels belonged to the SIT-tolerant group. Elevated tryptase levels

were not associated with specific allergens nor with distance from the specific pollen season. A bone marrow aspirate performed in the only patient with a history of systemic reactions following injection SIT and tryptase >11.4 ng/ml showed a normal morphology and phenotype. **Conclusions:** Unlike patients with hymenoptera venom allergy, in patients with respiratory allergy, elevated serum tryptase levels do not represent a risk factor for adverse reactions to SIT.

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Introduction

The detection of serum tryptase, an essential mast cell enzyme, is widely used in the diagnosis of mastocytosis or anaphylaxis. The association between mastocytosis and hymenoptera venom allergy has been recognized for some years, as well as the fact that venom-allergic patients with mastocytosis are at high risk for more severe allergic reactions [1, 2]. Recently, Bonadonna et al. [3] found a high prevalence of subjects with elevated baseline tryptase levels (in whom systemic or indolent mastocytosis was eventually diagnosed) and a history of severe systemic reactions to hymenoptera stings. Interestingly, this group of patients showed an increased incidence of ad-

verse side effects during immunotherapy with hymenoptera venom [4], and a recent study found that the measurement of baseline serum tryptase concentration before vespid venom immunotherapy is helpful to identify patients with a high risk of side effects [5]. In contrast to hymenoptera venom allergy, most allergic patients with a history of severe reactions induced by foods and drugs show normal baseline tryptase levels, and the association with mastocytosis is much less evident [6].

Allergen-specific immunotherapy (SIT) represents the only currently available treatment able to change the natural history of respiratory allergy [7]. Its effectiveness has been demonstrated by a number of properly performed studies and is now generally accepted. Injection SIT is generally considered a fairly safe procedure although systemic adverse reactions may occasionally occur [8]. In clinical practice, most patients undergoing injection SIT show excellent tolerance of the treatment and reach the scheduled high maintenance dose within a relatively short time and limited number of shots. However, a minority of patients with the same allergies, the same specific IgE levels and treated with the same commercial extracts quite inexplicably show recurrent systemic reactions following SIT shots; this may eventually lead to administration of extremely low maintenance doses or, sometimes, to withdrawal of the treatment. Since systemic allergic reactions following the injection of a SIT dose may sometimes resemble systemic reactions following hymenoptera stings in allergic subjects, we measured baseline tryptase levels in a cohort of atopic subjects, many of whom underwent injection SIT.

Patients and Methods

Patients

Baseline serum tryptase levels were measured in sera from 106 adults (38 males, 68 females; mean age 38.1 years, range 18–71 years) allergic to different airborne allergens attending the allergy centers of Milan and Paderno Dugnano and in sera from 40 normal, age- and sex-matched nonatopic subjects (13 males, 27 females; mean age 38.7 years, range 18–58 years).

Thirty-one allergic subjects (17 males, 14 females; mean age 43.5 years, range 19–73 years) had undergone injection SIT; 15 of these were selected because they had experienced at least one systemic reaction following injection immunotherapy shots during the SIT course, whereas the remaining 16 had tolerated SIT well. Some patients had experienced repeated adverse reactions that hampered the up dosing of the SIT. A systemic reaction was defined as the occurrence of urticaria with or without angioedema, asthma, gastrointestinal symptoms and/or hypotension after the subcutaneous administration of a depot aluminium hydroxide- or calcium phosphate-absorbed airborne allergen extract in subjects

who were well and showed a normal clinical examination before the injection. Most systemic reactions were immediate (within 30 min); however, in view of a recent study showing a high prevalence of delayed systemic reactions following injection SIT, reactions occurring within 6 h after the shot were also considered [8]. The severity of adverse reactions was graded based on the symptoms and response to rescue medication, according to the European Academy of Allergy and Clinical Immunology classification [9] revised by the World Health Organization [10] with some modifications, as follows: grade 0 = no symptoms; grade 1 = nonspecific symptoms, probably non-IgE-mediated; grade 2 (mild) = mild rhinitis or asthma, or mild urticaria responding to antihistamines or β_2 bronchodilators; grade 3 (non-life-threatening) = severe urticaria, angioedema and/or severe asthma that responds to treatment; grade 4 (anaphylactic shock) = rapidly evoked reaction including at least 2 out of skin (itching, flushing, erythema, urticaria, angioedema), respiratory (bronchial obstruction, rhinoconjunctivitis), cardiovascular (hypotension or collapse) and/or gastrointestinal symptoms requiring intensive treatment. All immunotherapies were administered following a perennial schedule with a coseasonal reduction by 50% of the maximum tolerated dose in the case of pollen vaccines. Weekly injections were given during the build-up phase; maintenance shots were given 3 weeks apart. In the case of systemic adverse reactions, the vaccine dose was suitably reduced. Appropriate rescue treatments were given to patients experiencing adverse reactions; those experiencing repeated adverse reactions were not given premedication. All adverse reactions occurred out of the pollen season, during either the buildup or the maintenance phase (table 1). All 15 patients were otherwise healthy without a history of urticaria and/or angioedema episodes before those induced by SIT. The remaining 16 allergic patients showed excellent tolerance of the SIT treatment throughout despite the use of the same therapeutic schedule and experienced only the usual local reactions at the site of injection. These 16 subjects along with the remaining 75 atopic subjects not submitted to SIT represented the 'positive control' group.

Tryptase Measurement and Statistics

Tryptase was measured by an immunofluorimetric assay (ImmunoCAP 250 Phadia, Uppsala, Sweden). In patients, measurements were made out of the pollen season and during a period of well-being. Although levels exceeding 11.4 ng/ml are usually considered to identify patients with possible mastocytosis among hymenoptera venom reactors [3], in this study, the absolute tryptase levels found in patients and controls were compared nonparametrically by Mann-Whitney U test. Proportions were compared by the χ^2 test with Yates' correction. The correlation coefficient (r) was calculated according to Pearson. Probability values $<5\%$ were considered statistically significant.

Results

Median baseline serum tryptase levels did not differ between atopic patients and normal controls [2.98 ng/ml (range 0–20.3) vs. 3.13 ng/ml (range 1.0–5.7), respectively; not significant], although levels exceeded 11.4 ng/ml in 6 of 106 patients (6%) versus 0 of 40 controls (0%).

Table 1. Clinical features of the study population

No.	Age years	Sex	Tryptase ng/ml	Allergy	SIT-induced adverse reactions	Timing of reactions	Severity
1	39	M	14.2	A	multiple episodes of urticaria and asthma	BP (10) + MP (25, 30)	3
2	31	M	3.1	A	urticaria and rhinitis (2 episodes)	BP (11, 12)	2/3
3	41	M	2.7	poly	some episodes of urticaria or asthma	BP (13) + MP (35)	2
4	48	F	3.7	A	some mild episodes of urticaria	BP (11, 13) + MP (21, 30, 55)	2
5	28	F	4.3	poly	multiple episodes of asthma or urticaria	BP (12, 18, 19) + MP (26, 29, 50)	2/3
6	33	F	5.4	A	some delayed episodes of urticaria	BP (11) + MP (23)	2
7	54	M	2	A	several episodes of mild urticaria	BP (8, 9, 14) + MP (26, 46)	2/3
8	49	F	2.4	poly	several episodes of urticaria	BP (9, 16) + MP (23, 24, 27, 32, 33, 57)	2
9	48	F	7	A	urticaria (1 episode)	MP (40)	2
10	60	M	6.4	A	several episodes of urticaria and asthma	MP (32, 39)	2/3
11	52	F	3.8	A	1 episode of anaphylaxis ¹	MP (44)	4
12	19	M	2.6	poly	several episodes of urticaria	BP (7, 9, 23) + MP (42)	2
13	50	M	5.8	A	some episodes of mild urticaria	BP (14) + MP (44)	2
14	60	F	3.4	Mt/A	urticaria (1 episode)	BP (24)	2
15	54	M	3.4	A	urticaria and asthma (2 episodes)	BP (14, 20)	2/3
16	48	F	1.6	A			
17	49	M	3.96	B			
18	38	F	4.3	poly			
19	73	M	7.35	A			
20	66	M	6.33	poly			
21	67	F	9.37	poly			
22	52	F	12.5	A			
23	47	M	13.2	G/A			
24	33	F	3.87	G			
25	45	F	6.68	G			
26	25	M	5.35	A			
27	44	M	5.51	B/C			
28	47	M	6.31	A			
29	66	M	20.3	A			
30	33	F	2.28	A/B			
31	29	M	4.79	poly			

Patients 1–15 were SIT reactors, while patients 16–31 were SIT tolerant. The figures in parentheses indicate the number of immunotherapy doses after which the reaction(s) occurred. A = Ragweed; G = grass; C = cypress; B = birch; Mt = mugwort; poly = hypersensitive to >2 airborne allergens; BP = buildup phase; MP = maintenance phase.

¹ Rapid occurrence of generalized pruritus, slight asthma and severe gastrointestinal cramps that lasted for about 45 min and gradually disappeared after administration of high-dose intravenous corticosteroids, antihistamines and inhaled β -agonists.

The findings in the 31 patients submitted to SIT are shown in table 1. With regard to median baseline serum tryptase levels, no difference between patients with or without a history of systemic reaction induced by injection SIT was found [median tryptase 3.7 ng/ml (range 2–14.2) vs. 5.91 ng/ml (1.6–20.3), respectively; not significant]. Four patients showed elevated tryptase levels, but 3 of them belonged to the SIT-tolerant subgroup. The two subpopulations of patients showed the same median age (48 and 47 years, respectively) and the same gender distribution (males/females 8/7 and 9/7, respectively). A weak but statistically significant positive correlation be-

tween age and tryptase levels was observed in the allergic group as a whole ($r = 0.35$; $p < 0.05$), although a comparison between tryptase levels in patients aged more or less than 40 years did not reveal any statistically significant difference (median tryptase 5.8 vs. 4.3 ng/ml, respectively; not significant). No correlation between specific allergen hypersensitivity and tryptase levels was observed; similarly, no correlation between the distance between the tryptase measurement and the specific pollen season in individual patients was found. Among atopic subjects not submitted to SIT, only 2 showed slightly elevated tryptase levels (11.7 and 12.2 ng/ml, respectively).

In the only patient with a history of systemic reactions following injection SIT and whose serum level of tryptase exceeded the 11.4 ng/ml threshold, suggesting possible mastocytosis (patient 1; table 1), bone marrow biopsy with marrow aspirate was carried out. Upon histological examination, although the analysis did not include an evaluation of KIT mutations or a cytofluorimetric assay for CD25 expression, mast cells showed normal morphology and phenotype. Further, no mast cell aggregates were detected. Except for a higher frequency and, possibly, a slightly increased severity of SIT-induced adverse reactions, this patient did not differ clinically from the other SIT reactors.

Discussion

Baseline serum tryptase is frequently increased in patients with mastocytosis, and temporary elevations may follow anaphylactic episodes. Thus, the fact that most allergic patients included in this study showed normal baseline levels of serum tryptase is not surprising, as none of the patients had a history of recent anaphylaxis or showed clinical signs of mastocytosis. However, although the median baseline tryptase level did not exceed that found in a normal control population, 6% of patients showed tryptase levels exceeding the cutoff that is generally used to identify patients with mastocytosis among hymenoptera venom reactors. Elevated serum tryptase levels were not associated with a history of systemic adverse reactions to injection SIT. It has to be noted that only in 1 of 6 patients showing elevated tryptase levels did these exceed 20 ng/ml, a minor criterion for the diagnosis of mastocytosis.

The only patient with a history of both SIT-induced systemic reactions and elevated serum tryptase, who underwent bone marrow biopsy, showed a sort of 'enhanced releasability' from a mast cell population that was numerically within the normal range and morphologically as well as immunohistochemically normal. It is possible

that this is the case also in other atopic subjects showing elevated tryptase levels.

In venom-sensitized subjects, a nonclonal mast cell activation disorder has recently been described [3] and clinically characterized [11]. However, the notion that allergic triggers, including injection SIT, may induce anaphylactic episodes in atopic subjects with a putative nonclonal mast cell activation disorder seems questionable, as in the present study only a small proportion of SIT reactors showed increased tryptase levels, and a similar (or even greater) proportion of subjects with elevated serum tryptase was present among SIT-tolerant subjects. Thus, our findings show that in patients with respiratory allergy, elevated tryptase levels do not identify a subset at high risk for systemic adverse reactions following injection SIT, as is the case for venom-allergic subjects [3, 12]. By applying the proposed score for predicting mast cell clonality [11], patients studied here (including those with increased serum tryptase levels) had a low probability of having a clonal bone marrow mast cell population. Thus, it might be hypothesized that in venom-allergic patients, serum tryptase levels by themselves are not responsible for a higher risk of severe reactions following hymenoptera venom immunotherapy but probably reflect an increased mast cell burden of an underlying mastocytosis. In contrast, subjects with respiratory allergy seem to have a low probability of having a clonal mast cell disorder, which translates into a lower mast cell burden and, consequently, lower tryptase levels. In conclusion, the presence of an underlying clonal mast cell disorder and not tryptase levels by themselves might be the true predictive factor involved in adverse reactions to immunotherapy. Akim et al. [13] very recently included allergic disorders within diseases characterized by mast cell activation. This study shows that subjects characterized by increased serum tryptase levels are more numerous than previously thought and that most of those with this condition may appear otherwise normal and healthy.

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