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Usefulness of UniCAP-Tryptase fluoroimmunoassay in the diagnosis of anaphylaxis

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Key words: allergy diagnosis; anaphylaxis; tryptase fluoroimmunoassay.

Background: Serum tryptase level measured by RIA is the main *in vitro* tool to confirm the diagnosis of anaphylaxis.

Methods: Serum tryptase levels were determined by UniCAP-Tryptase fluoroimmunoassay (Pharmacia & Upjohn, Uppsala, Sweden), in 30 consecutive patients who presented at the emergency room with a clinical allergic reaction of less than 6-h duration to assess the value of this method in the diagnosis of anaphylaxis. Anaphylaxis was established by clinical criteria and by immunoallergic study. Baseline tryptase levels were determined 1 month later in 21 patients. The receiver operating curve (ROC) was used to establish the best cutoff point of tryptase levels to confirm the diagnosis of anaphylaxis.

Results: Seventeen patients were diagnosed with anaphylaxis. In this group, tryptase levels were higher than in the nonanaphylaxis group, composed mostly of patients with urticaria or angioedema ($P<0.001$). ROC established the best cutoff of tryptase levels at 8.23 ng/ml with a 94.12% sensitivity and 92.31% specificity, whereas the 13.5 ng/ml cutoff recommended by the manufacturers showed 35.29% sensitivity and 92.31% specificity. The reaction-tryptase/baseline-tryptase ratio was 2.85 in the anaphylaxis group and 1.29 in the nonanaphylaxis group.

Conclusions: Serum tryptase levels of >8.23 ng/ml by UniCAP-Tryptase fluoroimmunoassay identify anaphylaxis in patients with symptoms of less than 6-h duration. The usefulness of this determination is higher if baseline tryptase levels are available.

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Tryptase, histamine, chymase, and other proteins are released from mastocytes in the course of immediate allergic reactions (1). Thus, tryptase or histamine serum levels have been used as markers of sudden mastocyte degranulation (2).

The special characteristics of tryptase, a protein which is highly selective for mastocytes (the low content of basophils is negligible) (3), is stable (4), and has a 2-h half-life period that permits serum levels to be detected for several hours (5), make it the ideal marker for anaphylaxis. The method of serum tryptase determination most used to date (RIACT Tryptase, Pharmacia, Uppsala, Sweden) has proved useful in the diagnosis of severe anaphylaxis, although its sensitivity is only moderate (2, 6–8). Recently, a new fluoroimmunoassay (UniCAP Tryptase, Pharmacia & Upjohn), capable of measuring both inactive (monomers) and active (tetramers) forms of the two types of tryptase (α - and β -tryptase), has been developed (9). Improved sensitivity is the main advantage of this new assay, since it can measure even the low serum tryptase levels of healthy subjects due to the mastocyte turnover or subclinical releases of the protein (9).

The aim of this study was to assess the usefulness of UniCAP Tryptase for identifying episodes of anaphylaxis in patients attending the emergency room with clinical symptoms of allergic reaction.

Material and methods

Thirty consecutive patients with symptoms of immediate allergic reactions of less than 6-h duration who attended the emergency room of two general hospitals were studied. Sera were obtained, centrifuged, and stored at -20°C . Patients were discharged after treatment and were referred within 2 weeks to an allergy section, where detailed clinical history, physical data, measurement of complement proteins and activity antinuclear antibodies (ANA), and skin tests to aeroallergens, foods, and drugs involved were recorded. Objective data of the clinical sheet from the clinical reaction day were scored, considering possible involvement of five different areas: the bronchial tree, the oropharynx, subcutaneous tissue and/or skin, the gastrointestinal tract, and the cardiovascular system. All patients in whom clinical data recorded sudden onset of symptoms plus simultaneous involvement of two or more of the five areas mentioned were diagnosed with anaphylaxis.

A second blood sample was collected in 21 patients 1–2 months after the clinical reaction for baseline tryptase level assessment. All samples were processed in the same assay by UniCAP-Tryptase fluoroimmunoassay according to the manufacturer's instructions.

Mean and standard deviations were calculated and the results processed by the nonparametric Mann–Whitney U and Wilcoxon tests. Statistical significance was established

for a value of $P < 0.05$. The receiver operating curve (ROC) which established the cutoff offering the best sensitivity and specificity for identifying anaphylaxis was chosen to study the usefulness of the assay.

Results

None of the patients required cardiorespiratory support or hospitalization. ANA were negative in all patients and none had abnormal C_3 , C_4 , or CH_{50} levels. Seventeen patients were diagnosed with anaphylaxis. Their clinical characteristics and tryptase levels, together with those of the 13 patients without anaphylaxis, are shown in Tables 1 and 2, respectively.

In the anaphylaxis group, serum tryptase levels in the acute episode were significantly higher than those of the nonanaphylaxis group (Table 3). In the anaphylaxis group, serum tryptase levels were higher in the clinical reaction compared with baseline samples, whereas no differences were found between the two samples in the nonanaphylaxis group or between baseline samples in both anaphylaxis and nonanaphylaxis groups (Table 3).

According to the manufacturer's instructions, only serum tryptase levels equal to or higher than 13.50 ng/ml are considered positive. With this cutoff, the assay sensitivity was 35.29%, with a confidence interval (CI) between 15.73 and 59.51%, whereas specificity was 92.31 (CI: 67.52–99.62%). With the ROC with a serum tryptase cutoff level of 8.23 ng/ml, sensitivity approached 94.12% (CI: 74.25–99.71%) and specificity 92.31% (CI: 67.52–99.62%), with an efficacy index of 93.2% (CI 82.90–100%), $P < 0.001$. No relationship was found between time elapsed from the beginning of the reaction to time of sampling and serum tryptase levels.

The reaction serum tryptase/baseline serum tryptase ratio, representing the increase in serum tryptase levels induced by the acute episode, was 2.85 ± 1.83 in the anaphylaxis group and 1.29 ± 0.62 in the nonanaphylaxis group. The ratio was always higher than 1.40 in patients with anaphylaxis.

Discussion

The diagnosis of anaphylaxis in emergency situations is based on clinical data, since confirmative biochemical tests were lacking until recently (2, 10). Diagnosis is more difficult in patients in whom cutaneous manifestations predominate, particularly when the causal agent is not

Table 1. Characteristics and serum tryptase levels of 17 patients with anaphylaxis

Patient no.	Age (years)	Sex	Causal agent	Symptoms	Serum tryptase (ng/ml)	
					Acute event	Baseline
1	18	F	Snail	B, AE	8.25	3.45
2	42	M	Idiopathic	B, U, L, H	11.60	6.95
3	65	M	Atracurium	U, H	11.80	7.29
4	41	M	Immunotherapy	B, U, H	10.80	5.77
5	36	F	Idiopathic	B, U, AE	12.90	5.91
6	21	M	Tomato	U, L, AE	9.04	6.12
7	27	M	Walnut	L, AE	9.32	1.52
8	44	M	Honey	B, R	8.29	4.16
9	33	F	Immunotherapy	B, U	6.15	3.58
10	27	M	Dipirone	B, U	16.00	2.41
11	36	F	Walnut	B, U, AE	8.48	1.88
12	47	F	Dipirone	B, U, H	19.30	9.50
13	66	F	Fish	B, U	33.10	ND
14	26	F	Idiopathic	L, U, AE, R	8.23	ND
15	40	F	Idiopathic	B, U	35.40	ND
16	79	F	Amoxicillin	U, H	16.90	ND
17	56	M	Cefuroxime	B, U, AE, H	61.30	ND

B: bronchospasm; U: urticaria; L: laryngoedema; AE: angioedema; H: hypotension; R: rhinitis; ND: not done.

Table 2. Characteristics and serum tryptase levels of 13 patients without anaphylaxis

Patient no.	Age (years)	Sex	Causal agent	Symptoms	Serum tryptase (ng/ml)	
					Acute event	Baseline
1	17	F	Scombroidosis	U	4.68	4.80
2	16	M	Scombroidosis	U	4.18	4.83
3	22	F	Dipirone	U	3.11	2.54
4	49	M	Idiopathic	AE	7.26	6.78
5	21	F	Idiopathic	AE	7.56	6.92
6	48	F	Chronic urticaria	U	14.20	6.30
7	31	M	Idiopathic	AE	5.97	3.30
8	31	M	Sulfites	B	5.39	4.80
9	28	F	Anxiety	HV	3.63	2.90
10	85	M	Idiopathic	AE	7.50	ND
11	7	M	Idiopathic	U	2.32	ND
12	23	M	Idiopathic	AE	6.32	ND
13	69	F	Unknown	EM	5.40	ND

U: urticaria; AE: angioedema; HV: hyperventilation; EM: erythema multiforme, ND: not done.

Table 3. Serum tryptase levels (ng/ml) in patients with and without anaphylaxis

	Anaphylaxis		Nonanaphylaxis	
	Acute episode	Baseline	Acute episode	Baseline
No. patients	17	12	13	9
Mean $\pm$ SD	16.87 $\pm$ 14.18 ^{a,b}	4.87 $\pm$ 2.43	5.96 $\pm$ 2.98	4.79 $\pm$ 1.64
Range	6.15–61.30	1.52–9.50	2.32–14.20	2.54–6.92
^a P<0.01 versus baseline.				
^b P<0.001 versus acute episode of nonanaphylaxis patients.				

evident, and many of these patients suffer recurrent episodes of undiagnosed anaphylaxis with a delay in the search for etiologic factors and establishment of proper treatment and/or preventive measures (11–13). In the present study, the search for a diagnosis in the 17 patients with anaphylaxis and high serum tryptase levels confirmed hypersensitivity in 13 (drug allergy in seven and food allergy in six); the remaining four patients were diagnosed with idiopathic anaphylaxis, whereas, in the nonanaphylaxis group, the diagnosis of allergy was confirmed in only one.

In this study, an increase in serum tryptase levels was observed in patients with recent onset anaphylaxis, whereas serum tryptase remained unchanged in the nonanaphylaxis group. This fact concurs with other studies where serum tryptase was measured by radioimmunoassay, suggesting that serum tryptase levels reflect quantitative degranulation of mastocytes and correlate with the intensity of the clinical picture (7, 8), and are undetectable in patients in whom only one site, generally the skin, is involved (14). On the other hand, the use of an 8.25-ng/ml cutoff point from the ROC improved sensitivity and specificity of the technique for detecting anaphylaxis, whereas baseline levels were similar

to those published in healthy populations (9). Only one patient, suffering from acute exacerbation of chronic idiopathic urticaria with extensive skin involvement, had an emergency room tryptase level higher than 8.25 ng/ml.

UniCAP-Tryptase, which permits measurement of active and inactive forms of both α and β types of tryptase, improves the sensitivity of anaphylaxis detection (9), although it should be remembered that the tryptase released in allergic reactions is mainly β -tryptase, whereas α -tryptase is a function of cell turnover, averaging 5 ng/ml in healthy subjects and rising mainly in systemic mastocytosis (15). We have no way of knowing what portions of α -tryptase or β -tryptase account for the total tryptase measured by the assay. Nevertheless, the data reported by Carjas et al. (16), who used old radioimmunoassay and new fluoroimmunoassay in patients with anaphylaxis, suggest that fluoroimmunoassay may improve anaphylaxis detection in some patients.

Individual comparison between baseline and reaction levels proved useful in our patients since the changes observed in the anaphylaxis group were significantly higher (twice baseline levels in most cases) than in the nonanaphylaxis group. These data concur with Schwartz et al., suggesting that the individual relative tryptase increment is more sensitive than the absolute value at the time of the clinical reaction (9). These and other authors have emphasized the slight intraindividual but wide interindividual variations in serum tryptase levels (7, 9).

Serum tryptase levels higher than 8.23 ng/ml measured by UniCAP-Tryptase are useful for detecting anaphylaxis. Usefulness improves if the value can be compared with baseline levels.

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