

# Usefulness and Limitations of Sequential Serum Tryptase for the Diagnosis of Anaphylaxis in 102 Patients

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## Key Words

Adrenaline · Epinephrine · Anaphylaxis · Emergency · Mast cell · Tryptase · Drug allergy · Food allergy

## Abstract

**Background:** The diagnosis of anaphylaxis is based on clinical history since no reliable biological marker is currently available to confirm the diagnosis. **Objective:** It was the aim of this study to determine sequential serum tryptase concentrations during anaphylaxis and to evaluate its potential as a diagnostic marker. **Methods:** We performed a prospective study including patients with acute anaphylaxis (according to the National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network criteria) attending the emergency department. Demographic characteristics, anaphylactic triggers, specific risk factors, clinical characteristics and management of anaphylaxis were recorded. Serum tryptase was measured at 1–2 h (T1), 4–6 h (T2) and 12–24 h (T3) following onset of the episode and at basal conditions (TB). **Results:** A total of 102 patients were included (63 females, mean age 47.4 ± 19.1 years). Tryptase concentration at T1 (19.3 ± 15.4 µg/l) was significantly high-

er than at T2, T3 and TB (all <11.4 µg/l; p < 0.0001). Importantly, tryptase was not raised in 36.3% of cases; furthermore, in 60.6% of these patients, no changes were observed in tryptase levels comparing T1 and TB ( $\Delta T1-TB = 0$ ). Tryptase was more frequently elevated in more severe anaphylaxis (p < 0.0001) and positively correlated with the grades of severity (p < 0.001, r = 0.49). Anaphylaxis was more severe and tryptase concentration higher when the causative agent was a drug compared to food, both at T1 (p = 0.045) and at TB (p = 0.019). Age and coronary risk factors were associated with more severe anaphylaxis (p = 0.001). **Conclusion:** Tryptase is a biomarker related to the severity of anaphylaxis. However, since its concentration remains unaltered in a considerable number of patients during acute anaphylaxis, there is a need for more reliable diagnostic biological tests.

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## Introduction

Diagnosis of anaphylaxis is based on suggestive clinical symptoms after exposure to a potential triggering agent or event [1, 2]. The clinical diagnosis may be sup-

**Table 1.** Grades of severity

Grade	Defined by
Mild <sup>1</sup> (skin and subcutaneous tissues only)	generalized erythema, urticaria, or angioedema
Moderate (features suggesting respiratory, cardiovascular or gastrointestinal involvement)	
A	nausea, vomiting or abdominal pain
B	dyspnea, stridor, wheeze, dizziness (presyncope), diaphoresis, chest or throat tightness
Severe (hypoxemia, hypotension or neurologic compromise)	cyanosis or SpO <sub>2</sub> ≤92% at any stage, hypotension (systolic blood pressure <90 mm Hg in adults), confusion, collapse, loss of consciousness or incontinence

Grades were adapted from the grading system for generalized hypersensitivity reactions by Brown [10]. SpO<sub>2</sub> = Oxygen saturation of hemoglobin, measured by pulse oximetry.

<sup>1</sup> The mild grade does not correspond with a diagnosis of anaphylaxis according to the criteria of the National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network.

ported by in vitro tests [1, 3]. Currently, plasma histamine (or its metabolite, methylhistamine in urine) and total serum tryptase are the only biomarkers available for routine use. The rationale for the use of these mediators for diagnosis is based on the fact that tryptase and histamine contained in mast cell granules are released upon activation of the cell [4]. However, their use in the diagnosis of an acute anaphylactic event has several limitations.

Plasma histamine peaks within 5–10 min of the onset of symptoms and declines to baseline within 60 min as a result of rapid metabolism by N-methyltransferase and diamine oxidase [4, 5]. Therefore, blood samples need to be obtained at the onset of the episode, which might only be possible in a small proportion of reactions but precludes it in most circumstances when reactions occur outside a hospital.

Nowadays, serum tryptase concentration is the most used laboratory test to confirm anaphylaxis. According to current knowledge, tryptase is the best biomarker to assess mast cell activation. Levels are increased from 15 min to 3 h after anaphylaxis onset [4, 6]. Although an elevated tryptase concentration supports this diagnosis, failure to document an elevation does not refute anaphylaxis. This is true even if the blood sample has been obtained adequately, and especially in cases of food-induced anaphylaxis where tryptase often remains low [7]. Serial measurements of total serum tryptase have been shown to increase the sensitivity and specificity of the test [8, 9]. Also, measurement of tryptase at baseline, obtained at least 24 h after resolution of symptoms, has been recommended in ascertaining whether or not anaphylaxis has occurred [8].

Therefore, the aim of our study was to determine sequential serum tryptase concentration in patients with anaphylaxis, both during the acute episode and at baseline, and to evaluate its usefulness in the diagnosis of anaphylaxis and as a marker related to the clinical severity of the reaction.

## Methods

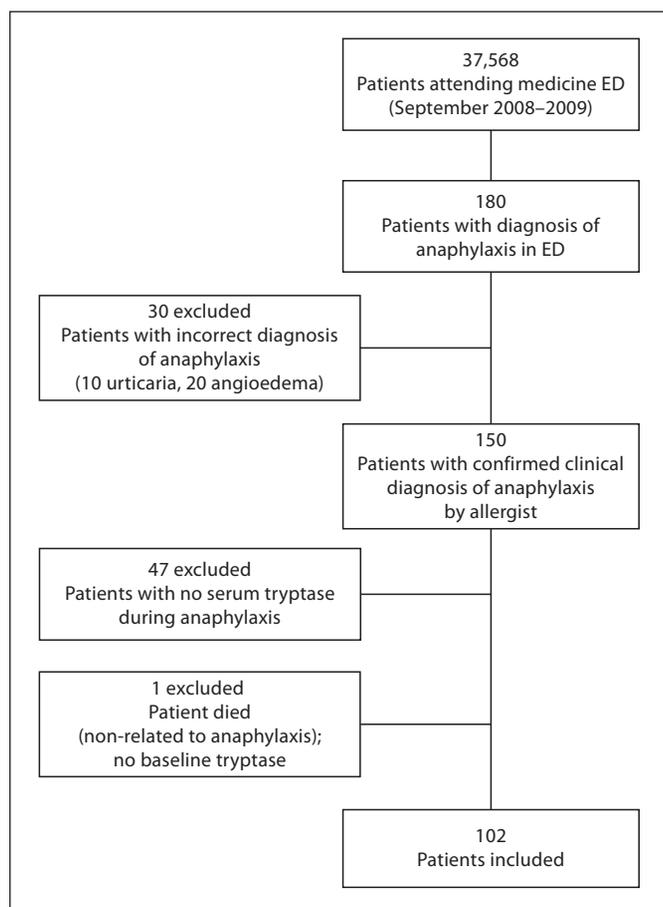
### *Study Population and Clinical Assessment*

An observational prospective study of cases of anaphylaxis was performed. All adult patients (≥18 years old) attending the medicine emergency department (ED) of the Hospital Universitari Vall d'Hebron, Barcelona, Spain, from September 2008 to September 2009 with a diagnosis of anaphylaxis were pre-included. Anaphylaxis cases were defined based on the criteria proposed at the 2006 National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network meeting [1]. Patients fulfilling these criteria, in which at least one tryptase serum concentration during the episode of anaphylaxis and one at baseline had been determined, were included. Patients were followed up at the allergy outpatient department where an allergological work-up was performed and the diagnosis of anaphylaxis was confirmed by an allergist.

Anaphylaxis severity was classified according to a grading system published by Brown [10] based on clinical symptoms (table 1). This classification was adapted and the moderate group was subdivided into group A, patients presenting with gastrointestinal symptoms, and group 2, patients presenting with respiratory or cardiovascular symptoms. Only patients with moderate or severe reactions were included for analysis.

Suspected triggers of anaphylaxis were registered using a detailed history of exposure in relation to the anaphylactic reaction. Conventional allergy diagnostic procedures were applied as needed (skin prick tests, specific IgE or challenge tests).

All patients signed an informed consent form on recruitment and the study was approved by the Hospital Ethics Committee.



**Fig. 1.** Study flow chart.

#### Serum Tryptase Concentration

Tryptase was measured using the UniCAP-Tryptase fluoroimmunoassay (Phadia, now Thermo Fisher Scientific, Uppsala, Sweden), following the manufacturer's instructions. The sensitivity of the assay is 1 µg/l. A serum tryptase concentration  $\geq 11.4$  µg/l was considered high [11].

Following the onset of symptoms, time points for tryptase measurement were: T1, 1–2 h; T2, 4–6 h; and T3, 12–24 h. A basal measurement was performed at least 1 week later when patients were completely asymptomatic (basal condition, TB).

Blood samples were kept at room temperature and centrifuged for 5 min at 2,500 g. Serum samples were stored at  $-20^{\circ}\text{C}$  until assessment of tryptase.

Two patients with indolent systemic mastocytosis were excluded from some analysis in order to avoid bias due to the persistently elevated tryptase concentrations.

#### Specific Risk Factors for Severity

Clinical risk factors for severity of anaphylaxis [12, 13] were evaluated: age, comorbidities, such as cardiovascular diseases, asthma or other chronic respiratory diseases, and mastocytosis. The use of concurrent medication, especially  $\beta$ -blockers, angio-

tensin-converting enzyme inhibitors (ACEi) and sedatives/hypnotics/antidepressants was documented [12].

#### Statistical Analysis

Data were collected and analyzed with SPSS version 17 (SPSS Inc., Chicago, Ill., USA) and Prism 5 (GraphPad Software, La Jolla, Calif., USA). Continuous variables are reported as the mean  $\pm$  SD, and exact 95% confidence interval (CI) is indicated. Categorical data were compared using the  $\chi^2$  and Fisher's exact test. Statistical analysis for comparison of tryptase concentration at different time points and between different etiologies was performed using paired and unpaired two-sided Student's *t* tests, respectively. Statistical analysis for comparison of tryptase concentration at T1 between different grades of severity was performed by using the non-parametric Kruskal-Wallis and Mann-Whitney *U* tests. A *p* value  $\leq 0.05$  indicates statistical significance.

## Results

#### Patient Characteristics

Out of 37,568 patients admitted to the medicine ED during the study period, 102 were finally included, with a mean age of  $47.4 \pm 19.1$  years (range 18–91; fig. 1). Patient demographics and clinical characteristics are outlined in table 2.

Thirty-nine patients had a personal history of allergic diseases, distributed as follows: rhinitis 32.4%, food allergy 13.7%, asthma 10.8%, anaphylaxis 4.9% and atopic dermatitis 1%. Only 5 patients (4.9%) reported a previous anaphylactic event. When analyzing food-induced anaphylaxis (35/102), only 11/35 patients (31.4%) had an established diagnosis of food allergy and recalled having had urticaria or oral symptoms, after previous consumption of the eliciting food; in the other 24/35 patients (68.6%), anaphylaxis was the first manifestation of the disease. In 98% of drug-induced events, anaphylaxis was the first manifestation.

#### Anaphylaxis Severity and Triggers

Anaphylactic triggers and severity grades are shown in table 3. Drug-induced anaphylaxis was significantly more frequent and more severe than anaphylaxis caused by food [severe grade in 32/51 patients (62.7%) vs. 10/35 (28.5%); *p* = 0.001].

#### Serum Tryptase Concentration

Only 63 of all 102 patients (61.8%) showed elevated tryptase concentration during anaphylaxis.

Tryptase concentrations and percentages of patients with elevated tryptase at different time points are shown in figure 2a and table 4, respectively. At T1, only 58 of 91

**Table 2.** Patient demographics and clinical characteristics

	n	%
Overall	102	100
Sex		
Male	39	38.2
Female	63	61.8
Age		
18–65 years	83	81.6
>65 years	19	18.4
Anaphylaxis severity		
Moderate	56	54.9
A	14	13.7
B	42	41.2
Severe	46	45.1
Etiology of anaphylaxis		
Drug	51	50
Food	35	34.5
Anisakis	5	4.9
Latex	3	2.9
Hymenoptera	3	2.9
Cold	1	0.9
Unknown	4	3.9
Family history of atopy	21	20.6
Personal history of atopy	39	38.2
Comorbidities		
Rhinitis	33	32.4
Cardiovascular diseases	32	31.4
Asthma and other respiratory diseases	13	12.7
Psychiatric disease	10	9.8
Neurologic disorders	4	3.9
Thyroid disease	3	2.9
Mastocytosis	2	1.96
Concurrent medication		
ACEi	18	17.6
Sedatives/hypnotics/antidepressants	9	8.8
β-Blockers	4	3.9
Other	50	49

**Table 3.** Etiologic agents according to severity of anaphylaxis

Grades of anaphylaxis	Moderate		Severe	Total patients
	A	B		
Etiology of anaphylaxis				
Drug	3	16	32	51 (50)
Food	8	17	10	35 (34.5)
Anisakis	2	2	1	5 (4.9)
Latex	0	2	1	3 (2.9)
Hymenoptera	0	1	2	3 (2.9)
Cold	0	1	0	1 (0.9)
Unknown	1	3	0	4 (3.9)
Total patients	14 (13.7)	42 (41.2)	46 (45.1)	102 (100)

Figures in parentheses are percentages.

**Table 4.** Serum tryptase at different time points and percentage of patients with elevated tryptase

	Mean, µg/l	SD, µg/l	Total patients <sup>1</sup>	n <sup>2</sup>
T1 (1–2 h)	19.3	15.1	58 (63.7)	91
T2 (4–6 h)	10.2	8.5	20 (46.6)	45
T3 (12–24 h)	7.3	4.7	9 (33.3)	27
TB (basal)	4.5	2	0	100 <sup>3</sup>

Figures in parentheses are percentages.

<sup>1</sup> Total number of patients with elevated tryptase (>11.4 µg/l) in each group.

<sup>2</sup> Total number of patients in each group.

<sup>3</sup> The 2 patients with mastocytosis were excluded from the analysis to avoid bias.

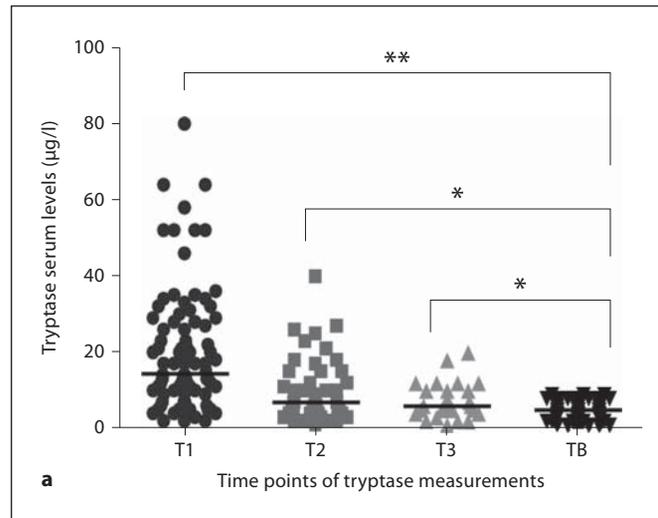
patients (63.7%) showed elevated tryptase and it was significantly higher than at any other time point: T1 versus T2 ( $\Delta$  5.4 µg/l, range 2.7–8.2;  $p < 0.001$ ) and T1 versus T3 ( $\Delta$  11.7 µg/l, range 7.3–16.1;  $p = 0.001$ ).

In the severe group, serum tryptase was elevated in 35/46 patients [76%; median 25.3, interquartile range (IQR) 17 µg/l], in the moderate grade subgroup B in 23/42 patients (55%; median 14.6, IQR 10.7 µg/l), and in the moderate grade subgroup A in 5/14 patients (36%; median 12.7, IQR 14.2 µg/l) with a statistically significant difference in median tryptase concentration between groups of subjects with anaphylaxis of severe and moderate A and B grade ( $p = 0.024$ ; fig. 3). Moreover,

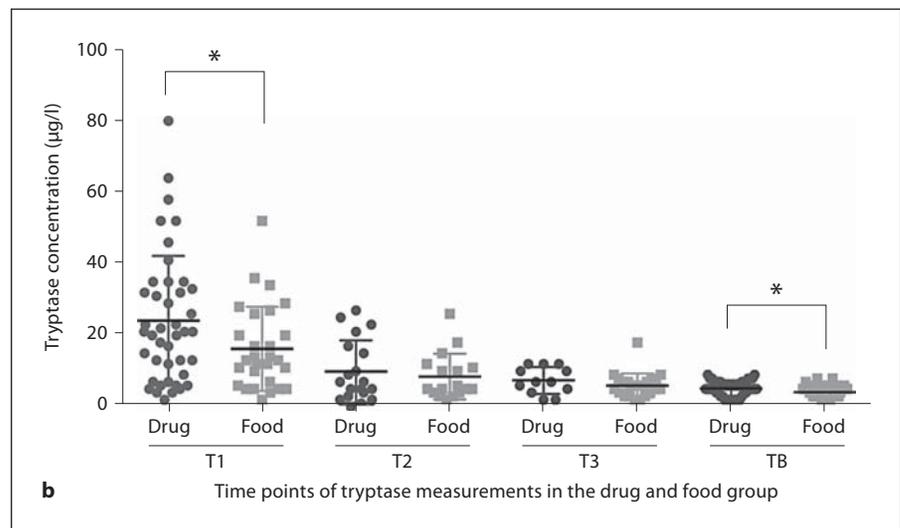
tryptase concentration was more frequently elevated in severe anaphylaxis, with a positive correlation between grades of severity and serum tryptase ( $p < 0.001$ ;  $r = 0.49$ ).

On the other hand, 36.3% of patients (33/91) showed normal tryptase concentration at T1 (<11.4 µg/l). In only 39.4% of these cases (13/33) was there a difference between T1 ( $5.9 \pm 3.2$  µg/l) and TB ( $3.1 \pm 2.5$  µg/l). Interestingly, in 60.6% of these patients (20/33) tryptase remained unaltered when comparing T1 and TB ( $\Delta$ T1–TB = 0).

There were no significant differences in the number of patients with elevated tryptase when the cause was drug



**Fig. 2. a** Tryptase concentration at different time points. Results are expressed as means. Differences in tryptase concentration: T1 versus TB, 14.4 µg/l (95% CI 11.2–17.6), \*\*  $p < 0.0001$ ; T2 versus TB, 6.3 µg/l (95% CI 3.9–8.6), \*  $p < 0.001$ ; T3 versus TB, 3.1 µg/l (95% CI 1.7–4.6), \*  $p < 0.001$ . **b** Tryptase concentration at different time points depending on the etiology: drug versus food. Results are expressed as means and SD. There were statistically significant differences (\*  $p < 0.05$ ) only in T1 and TB.



or food [65% (33/51) vs. 57% (20/35);  $p = 0.523$ ], but concentration at T1 was higher when the reaction was caused by drugs (mean  $24.14 \pm 18.08$  µg/l, 95% CI 18.51–29.78;  $n = 51$ ) than by food (mean  $16.25 \pm 11.76$  µg/l, 95% CI 11.69–20.81;  $n = 35$ ;  $p = 0.045$ ). There were no differences at other time points, except at TB when tryptase was also higher in the drug group compared to the food group [mean  $5.21 \pm 2.13$  µg/l (95% CI 4.58–5.82) vs.  $4.14 \pm 1.87$  µg/l (95% CI 3.51–4.76);  $p = 0.019$ ] (fig. 2b).

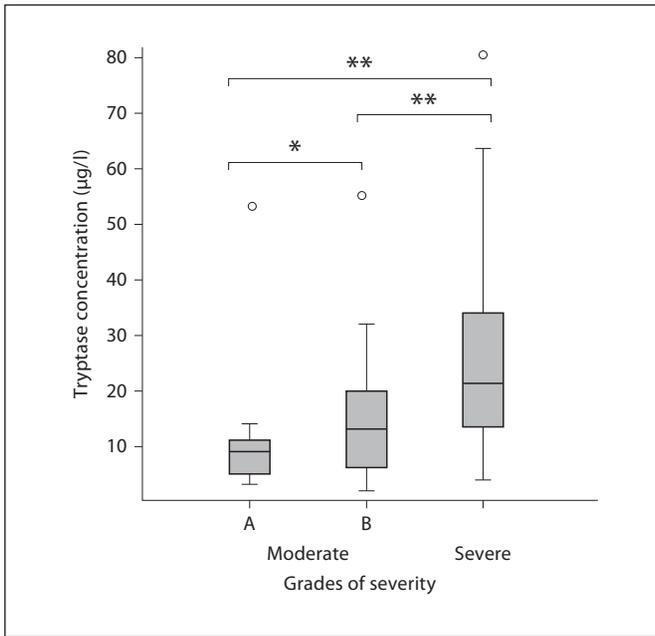
#### Specific Risk Factors for Severity

In patients with comorbidities, anaphylaxis was significantly more severe than in patients without a relevant past medical history [55.5% (30/54) vs. 33.3% (16/48);  $p = 0.024$ ].

Severe anaphylaxis was significantly associated with old age (>65 years), coronary risk factors and ACEi intake, but not with respiratory diseases or other medications (table 5). The 2 patients with indolent systemic mastocytosis presented a severe anaphylaxis.

#### Discussion

The present study demonstrates that although tryptase is considered a specific marker of mast cell degranulation [4, 8], it is not always elevated during anaphylaxis. In fact, in 36.3% of our patients with clinically defined anaphylaxis, tryptase remained low during the acute episode (T1).



**Fig. 3.** Tryptase concentration during anaphylaxis (T1) depending on severity. Results are expressed as the median and IQR. Kruskal-Wallis test among all groups,  $p < 0.001$ . Moderate-A versus moderate-B,  $* p < 0.05$ ; moderate-B versus severe,  $** p < 0.001$ ; moderate-A versus severe,  $** p < 0.001$ .

To our knowledge, this is the first study with such a large number of patients in a single center with a clinical syndrome consistent with anaphylaxis and with serial serum tryptase determinations during an acute episode, which evaluates tryptase concentration depending on severity, etiology (drug vs. food) and risk factors for severity. A previous large multicentric study by Stone et al. [14] including 78 patients evaluated serial tryptase determinations and found that a non-negligible percentage of patients (36%) did not have elevated tryptase during acute anaphylaxis. Nevertheless, they recommend to assess the increase in tryptase compared to baseline ( $\Delta T1-TB$ ), as they did find differences even within normal tryptase concentration.

In our study, even though all the reactions were consistent with anaphylaxis and blood had been obtained at different time points, there was also a considerable percentage of patients in which tryptase remained normal during anaphylaxis (almost two thirds) even when anaphylaxis was severe. This is in contrast with the assumption that in many patients the difference or the ratio of tryptase during anaphylaxis and at baseline might aid in the diagnosis of anaphylaxis, as suggested by Brown and

**Table 5.** Patient factors associated with anaphylaxis severity

	n <sup>1</sup>	Patients with severe anaphylaxis	Effect p value
Age $\geq 65$ years	19/102	16 (84)	<b>0.002</b>
Coronary risk factors	32/102	23 (72)	<b>0.001</b>
Respiratory disease	13/102	6 (46.1)	0.935
Rhinitis	33/102	7 (21.2)	0.939
Concurrent medication			
ACEi	18/102	15 (83.3)	<b>0.001</b>
$\beta$ -Blockers	4/102	2 (50)	0.325
Sedatives/hypnotics/ antidepressants	9/102	4 (44.4)	0.613

Figures in parentheses are percentages. Boldfaced values indicate  $p < 0.05$ .

<sup>1</sup> The number of patients with each risk factor among all patients.

Stone [15]. Therefore, neither tryptase concentration during acute anaphylaxis nor the difference between anaphylaxis and baseline were useful for the diagnosis in 20 of 91 patients (21.9%).

Previous studies recommend obtaining serum samples within 3 h of onset of symptoms in order to measure tryptase [9]. Our results suggest that the window of opportunity for a good diagnostic sensitivity of tryptase is within the first 2 h, rapidly decreasing after that.

Lin et al. [16] published a study in which a single determination of histamine and tryptase in patients with acute allergic reactions was evaluated. The majority of cases presented mild reactions (such as urticaria) and only a few had systemic anaphylaxis. Histamine was more frequently elevated than tryptase and, according to the authors, this could be attributed to the inclusion of mild reactions [14]. In fact, some studies on drug-induced anaphylactic reactions suggest that milder allergic reactions are associated with histamine and not tryptase increase [17]. In another study of food-associated severe anaphylaxis in children and adolescents, Sampson et al. [7] did not find tryptase elevations in the 2 patients whose serum was available. The hypothesis is that some anaphylaxis may primarily involve basophils rather than mast cells. Other authors have described that anaphylaxis induced by food infrequently elevates tryptase levels [18, 19]. Presumably, this is due to a localized rather than a generalized mast cell degranulation, rendering a small amount of tryptase entering the circulation too small to raise serum levels [19, 5]. In accordance with these find-

ings, we have observed that in anaphylaxis induced by food, only 57% of cases presented elevated tryptase. Although the percentage was very similar in drug-induced reactions, the mean concentration of tryptase in these cases was significantly higher.

A number of severity grading systems of anaphylaxis have been described [20–26]. We use the clinical grading system developed by Brown [10], which was later used by Stone et al. [14] who found a correlation between severity grades and tryptase concentration at different time points. However, we subdivided the moderate group into 2 subgroups (groups A and B) depending on the symptoms of the patients. We performed this subanalysis to confirm our observation that patients with gastrointestinal symptoms (moderate-A) presented lower levels of serum tryptase than patients with respiratory or cardiovascular symptoms (moderate-B). This severity grading correlated with levels of serum tryptase, which is in line with the fact that gastrointestinal mast cells predominantly contain chymase over tryptase. Thus, our study replicates the findings by Stone et al. [14], even when using the modified grading system that provides more refined information.

As described in the literature, elderly adults are at increased risk of fatality during anaphylaxis for a variety of reasons [12, 27], including concomitant diseases [28, 29], such as respiratory conditions, cardiovascular disease, and the use of concurrent medications such as ACEi and  $\beta$ -adrenergic blockers [6, 12, 29, 30]. In our study, older age and the presence of cardiovascular disease was associated with more severe anaphylaxis, highlighting the importance of these risk factors during an episode of anaphylaxis. On the contrary, we have not found significant association between rhinitis or respiratory disease and the severity of anaphylaxis, although these patients tended to develop respiratory involvement more frequently.

Despite some authors having recognized concurrent medication, such as  $\beta$ -adrenergic blockers, ACEi and antidepressants, as being associated with severe reactions and death [12, 31, 32], in our study, only ACEi were associated with severe reactions. This may be the case because the size of each group is small; another plausible explanation is that patients on ACEi are often older and affected by cardiovascular disease, so these could be confounding factors.

Methodological limitations inherent to the study design must be pointed out. The first is a selection bias. We included patients with anaphylactic reactions attending the ED of a tertiary university hospital in which tryptase had been determined; therefore, severity groups are not homogeneous, as the moderate-A group is underrepre-

sented and the great majority of anaphylaxis was moderate-B or severe. Possible explanations are that patients referred to our ED presented more severe symptoms, since this is a referral center, or that milder reactions were not recognized or managed as anaphylaxis. A further confusion bias in assessing risk factors for severity of anaphylaxis, such as age, is that a high number of these patients have coronary risk factors and are on ACEi, also considered risk factors for severe anaphylaxis. Finally, although the clinical diagnostic criteria used have been shown to perform well [33], having no gold standard to ascertain the diagnosis of anaphylaxis, some cases may have been falsely diagnosed.

In conclusion, tryptase is not an optimal marker for the diagnosis of anaphylaxis, since although the concentration of tryptase correlates with severity, levels are not increased in a considerable number of patients during acute anaphylaxis. Therefore, further studies are needed in order to identify additional sensitive markers to support the clinical diagnosis of anaphylaxis.

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### Disclosure Statement

The authors have no conflict of interest to declare.

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