

Diagnostic Value of Tryptase in Food Allergic Reactions: A Prospective Study of 160 Adult Peanut Challenges



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What is already known about this topic? Serum tryptase has been shown to be useful in confirming anaphylaxis caused by venom or drugs; however, its utility in food allergic reactions is unknown.

What does this article add to our knowledge? This prospective study shows that tryptase rises acutely compared with baseline levels during peanut allergic reactions. Peak levels occur at 2 hours and correlate with severity. An optimal cutoff in tryptase rise of 30% is established which identifies an allergic reaction.

How does this study impact current management guidelines? Serum tryptase measurement is valuable in food allergic reactions. The cutoff in tryptase rise may guide clinicians in establishing whether mast cell activation in a food allergic reaction has occurred in both an emergency and challenge setting.

BACKGROUND: Serum tryptase is useful in diagnosing drug and venom anaphylaxis. Its utility in food anaphylaxis is unknown.

OBJECTIVE: The objective of this study was to determine whether tryptase rises in food allergic reactions, optimal sampling time points, and a diagnostic cutoff for confirming a clinical reaction.

METHODS: Characterized peanut allergic patients were recruited and underwent up to 4 peanut challenges and 1 placebo challenge each. Tryptase was measured serially on challenge days both before (baseline) and during the challenge. The peak percentage tryptase rise (peak/baseline) was related to reaction severity. Receiver operating characteristic (ROC) curves were generated establishing an optimal diagnostic cutoff.

RESULTS: Tryptase was analyzed in 160 reactive (9% anaphylaxis) and 45 nonreactive (placebo) challenges in 50 adults aged 18 to 39 years. Tryptase rose above the normal range (11.4 ng/mL) in 4 of 160 reactions. When compared with baseline levels, a rise was observed in 100 of 160 (62.5%)

reactions and 0 of 45 placebo challenges. The median rise (95% confidence interval [CI]) for all reactions was 25% (13.3% to 33.3%) and 70.8% (33.3% to 300%) during anaphylaxis. Peak levels occurred at 2 hours and correlated with severity ($P < .05$). Moderate-to-severe respiratory symptoms, generalized erythema, dizziness, and hypotension were correlated with a higher peak/baseline tryptase ($P < .05$). ROC curve analysis demonstrated the optimal cutoff to identify a reaction as a 30% rise (sensitivity 0.53; specificity 0.85), area under the curve 0.72 (95% CI, 0.67-0.78).

CONCLUSIONS: Serum tryptase measurement is valuable in food allergic reactions, and correlates with symptom severity. Comparing peak reaction levels at 2 hours with baseline is essential. A rise in tryptase of 30% is associated with food allergic reactions. Crown Copyright © 2018 Published by Elsevier Inc. on behalf of the American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2018;6:1692-8)

Key words: Peanut allergy; Anaphylaxis; Adults; Tryptase; Diagnosis

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Serum tryptase is a marker of mast cell activation, and a rise in tryptase is a useful indicator of whether an allergic reaction has taken place. Tryptase has been shown to be of value in allergic reactions triggered by venom¹ and drugs²; however, its utility in food allergic reactions remains unknown. Some studies report that tryptase does not rise in food-induced anaphylaxis³ nor in nonhypotensive reactions. These studies are largely based on data derived from postmortem samples of patients who died from anaphylaxis⁴ or patients presenting acutely to emergency departments resulting in varying sample times and a bias toward more severe reactions. Experimental evidence of tryptase in food allergic reactions and in particular on its typical time course is lacking.

Abbreviations used

CI- Confidence interval

IQR- Interquartile range

ROC- Receiver operating characteristic

SPT- Skin prick test

We present a large prospective study of the diagnostic utility of serum tryptase in experimentally induced peanut allergic reactions of varying severities. Our aim was to establish whether tryptase rises in food allergic reactions, the optimal time point for tryptase sampling, and an optimal diagnostic cutoff in serum tryptase rises for determining a reaction versus no reaction.

METHODS

We carried out a prospective study of mast cell tryptase during peanut allergic reactions.

Patients

Adult patients with a history of systemic reactions (urticaria, angioedema, respiratory/gastrointestinal tract symptoms, and anaphylaxis), with acute onset of symptoms after ingestion (up to 2 hours) of peanut, were recruited from 2013 to 2016. Allergic status was determined by evidence of sensitization to peanut demonstrated by positive skin prick tests (SPT) and positive serum specific IgE using ImmunoCAP (ThermoFisher, Uppsala, Sweden) to peanut and peanut components Arah 2 and/or 1 and 3. A positive test was defined using the criteria of ≥ 3 mm for SPT and ≥ 0.35 kU/L for ImmunoCAP. All patients underwent an initial peanut challenge. The patients were given incrementally increasing doses of peanut protein until they developed clear objective signs of an allergic reaction. They were given increasing doses of masked food matrix containing peanut flour. Doses were delivered at 30-minute intervals starting with an initial dose of 3 μ g to a total cumulative dose of 1,433 mg of peanut protein. Reaction severity was scored according to the Ewan and Clark severity scale.⁵ Anaphylaxis was defined as 2 or more of the following rapidly occurring symptoms (minutes to hours) after exposure to peanut: generalized involvement of the skin-mucosal tissue, respiratory compromise (pronounced dyspnoea, wheeze, bronchospasm), reduced blood pressure or associated symptoms, severe and persistent abdominal symptoms (cramping abdominal pain and vomiting),⁶ and corresponded to Ewan and Clark severity grade 5 reactions. The first peanut challenge was double blind with all patients undertaking a placebo arm. In the placebo arm, patients were given the same masked food matrix without allergen. The initial challenge was followed by 3 further open challenges. Ethical approval was obtained from the National Research Ethics Service Committee East of England. Informed consent was obtained from all patients.

Mast cell tryptase

Blood samples for mast cell tryptase were taken on each challenge day before the commencement of peanut doses (prechallenge sample, at the onset of reaction, and at 1 and 2 hours after the reaction). On nonreactive challenge days, samples were taken before the commencement of doses and at 2 hours after the last dose. An initial serum tryptase was also taken for each patient on his or her screening visit. Tryptase was measured using UniCAP (Thermo Scientific, Uppsala, Sweden). The detection limit of the assay was 1 ng/mL. The upper limit of normal was taken to be 11.4 ng/mL (95th centile).

Statistical analysis

Mean and standard deviations were calculated, and because of nonnormality, median and a nonparametric confidence interval (CI) around the median were used. A *P* value of $<.05$ was classed as statistically significant. The peak percentage rise was calculated as follows: (peak tryptase level during reaction/same day prechallenge sample) $\times$ 100. Correlations were calculated. To investigate the utility of serum tryptase in determining whether an allergic reaction has occurred, receiver operating characteristic (ROC) curves were used to establish the cutoff providing the best sensitivity and specificity for the test. Statistical analyses were performed with STATA version 12 (StataCorp, College Station, Tex).

RESULTS

Patient characteristics

Fifty adults aged 18 to 39 years (median age 20.8, *M* = 28, *F* = 22) were included in the study. Tryptase readings were measured in 177 reactive peanut and 45 nonreactive challenges. In 17 of 177 challenges, tryptase levels were <1 ng/mL and therefore were unable to be analyzed. All patients were reactive and developed objective symptoms during the peanut challenges. Five patients developed mild, transient subjective symptoms during placebo challenges; however, no patient developed objective symptoms. Patients were therefore deemed nonreactive during the placebo challenges. Fourteen of the 160 reactions (9%) were classed as anaphylaxis. No patients had a raised basal tryptase level.

Tryptase during placebo challenges

Tryptase readings were available for 45 placebo challenges. In the placebo challenges, the percentage change of the 2-hour reading/baseline was zero in 21 cases, positive in 11, and negative in 13. The median peak percentage rise was zero (95% CI, 0, 0; interquartile range [IQR] -1.92 , 4.31), the arithmetic mean (which gives higher weight to proportional increases than decreases) was 1.4%, and the geometric mean (which gives both proportional increases and decreases equal weight) was -0.1% . Patients with higher placebo rises did not exhibit higher reaction rises.

Tryptase rise during reactions

Tryptase was raised above the upper limit of the normal range (11.4 ng/mL) in 4 of 160 reactions, and all 4 reactions were severe anaphylaxis. The predominant symptom was hypotension in 1 of 4 reactions and profound dyspnoea with no hypotension in 3 of 4 reactions. Peak levels were 17, 13, 12.2, and 12 ng/mL, respectively (Table E1, available in this article's Online Repository at www.jaci-inpractice.org). In 10 of 14 anaphylactic reactions, the peak tryptase remained in the normal range. The peak percentage rise was calculated for all reactions. Relative to the baseline, a rise was noted in 100 of 160 reactions (62.5%), anaphylaxis group 14 of 14 (100%), and nonanaphylaxis group 86 of 146 (59%). The average peak percentage rise for all reactions was 34.8% (median 25%, 95% CI, 13.3% to 33.3%). In the anaphylaxis group, the average rise was 148.4% (median 70.8%, 95% CI, 33.3% to 300%), and in the nonanaphylaxis group, 23.9% (median 14.0%, 95% CI, 0% to 25%).

Time course of serum tryptase during reactions

Median percentage change in tryptase over time is shown in Figure 1 (A, B). Relative to a completely flat time course in placebo challenges, tryptase levels rose over time in positive

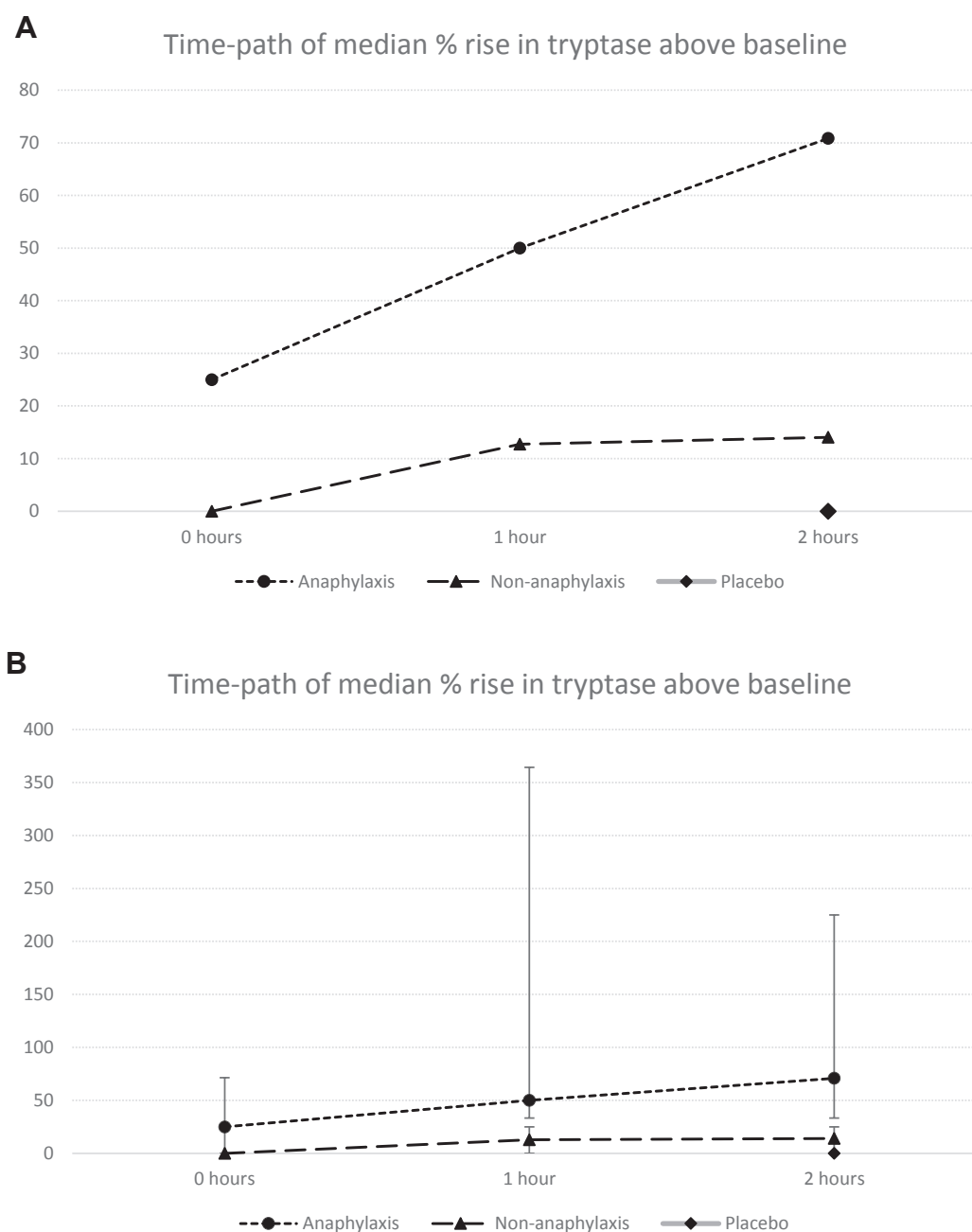


FIGURE 1. A, Tryptase time course during peanut and placebo challenges. Median percentage rise in tryptase (peak tryptase/baseline) for each time point is shown. B, With confidence intervals.

challenges. Tryptase followed a steeper time course in anaphylaxis reactions versus nonanaphylaxis reactions. The commonest time for peak values to occur in both groups was 2 hours (57% anaphylaxis group and 36.5% nonanaphylaxis group). The same peak readings at both 1 hour and 2 hours were observed in 35.7% of patients in the anaphylaxis group and 27.1% of patients in the nonanaphylaxis group. No patient experienced a peak level independently at 0 hours (onset of reaction) (Table I).

Tryptase in relation to severity

Using the Ewan and Clark severity grading system the reactions were graded as shown in Table II. The peak percentage

rise in tryptase was regressed on severity grade using dummy variables. Average peak tryptase increased successively with severity grade: 9.9% for grade 2, 24.9% for grade 3, 26.2% for grade 4, and 163.7% for grade 5. The increase was statistically significant between grades 2 and 3 ($P < .00$) and 4 and 5 ($P < .00$) but not 3 and 4 ($P = .79$) (Figure 2).

Clinical features associated with a rise in tryptase

Correlations were measured between the peak percentage rise in tryptase and binary symptom variables (localized cutaneous erythema, generalized erythema, mild respiratory symptoms, moderate-to-severe respiratory symptoms, abdominal

TABLE I. Timing of peak readings (% patients in each group)

	0 h	1 h	2 h	1 h/2 h joint*	0 h/1 h joint	0 h/1 h/2 h joint	0 h/2 h joint
Anaphylaxis	0.0	7.1	57.1	35.7	0.0	0.0	0.0
Nonanaphylaxis	0.0	16.5	36.5	27.1	3.5	14.1	2.4

*Joint denotes when the same peak reading was observed at multiple sample times.

TABLE II. Frequency and classification of systemic reactions (n = 160) during peanut challenges according to Ewan and Clark Severity Grade

Severity grade	Symptoms	Frequency (%)
1	Localized cutaneous erythema/urticaria/angioedema/oral pruritus	0
2	Generalized erythema/urticaria/angioedema	3.1
3	At least grade 1 or 2 and GI symptoms/rhinoconjunctivitis	42.5
4	Mild laryngeal edema voice change/tightening of throat/mild asthma	46.9
5	Severe pronounced dyspnoea/hypotensive symptoms/collapse/loss of consciousness	7.5

GI, Gastrointestinal.

pain/nausea, vomiting, throat tightness, hypotension, and dizziness/tachycardia with no hypotension). Statistically significant positive correlations ($P < .05$) were found with generalized erythema, moderate-to-severe respiratory symptoms, hypotension, and dizziness.

Within patient variation in baseline tryptase over time

Inter-day within-patient variation of baseline was summarized by averaging measures of within-patient variation across all patients. Two hundred and fifty-nine baseline samples were included in the analysis: 50 screening, 160 on the day before challenge, and 49 placebo baseline samples. The average patient mean baseline was 3.74 ng/mL, based on an average of 5 observations. The average patient standard deviation of baseline was 0.46 ng/mL, giving an average coefficient of variation of baseline of 0.14 corresponding to a baseline variation of 14%. The average IQR of baseline was 0.59 and the average max-min range was 1.08.

Relationship of baseline tryptase levels to reaction severity

No correlation was found between a patient's median baseline tryptase level and the grade of their most severe reaction (correlation coefficient -0.09 , $P = .54$). No baseline tryptase was raised >11.4 ng/mL.

Clinical utility of serum tryptase as a diagnostic marker

An ROC analysis was performed to identify the optimal cutoff point for the percentage rise in tryptase above baseline to distinguish between a reaction and no reaction in our population.

A clinician measuring tryptase acutely during a suspected reaction may compare this reading with a baseline taken at clinic either on a previous occasion or during subsequent assessment. The diagnostic usefulness of the acute reading depends on whether the difference between reaction and nonreaction (baseline) readings is large relative to inter-day variation in baseline readings.

Thus the following 2 distributions were compared in the ROC analysis: (1) peak tryptase rise (160 reaction observations)

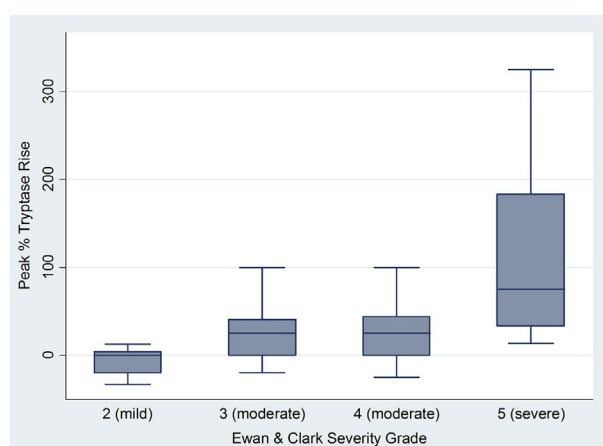


FIGURE 2. Serum levels of tryptase as a function of the severity of the allergic reaction.

relative to screening baseline and (2) subsequent day baselines relative to screening baseline (209 observations) the distributions of which are shown in Figure 3. The 209 subsequent baseline observations are clustered around zero and symmetric, whereas the reaction observations exhibit far fewer zeros and are markedly skewed positive.

Combining these 369 observations, the area under the ROC curve was 0.72 (95% CI, 0.67-0.78), indicating that the percentage tryptase difference relative to a different-day baseline is potentially useful as a test to identify the presence of a reaction in a clinical context. The Youden index, which weights the cost of false positives and false negatives equally, identified the optimal cutoff to further identify a reaction as a 30% rise, which was associated with a sensitivity of 0.53 and specificity of 0.85 (Figure 4). As an alternative approach to identifying an appropriate cutoff, taking the 2 standard deviation within-patient percentage variation in baseline above the patient-specific mean baseline (which represents a 95% upper confidence bound on percentage baseline variation) yields a cutoff of 28%, which is close to the 30% derived from the ROC analysis. Taking the median baseline value of tryptase for the median subject

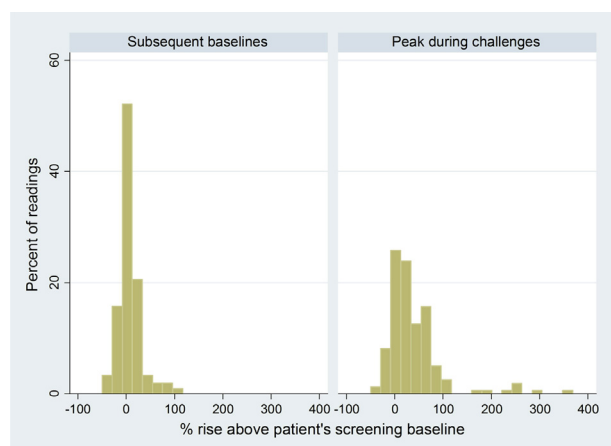


FIGURE 3. Histogram demonstrating percentage change relative to initial baseline in reaction versus no reaction.

(4 ng/mL), a 30% rise equates to a 1.2 ng/mL increase in serum tryptase to 5.2 ng/mL.

DISCUSSION

We have conducted a large prospective study on serum mast cell tryptase during 177 experimentally induced peanut allergic reactions. We provide new data on tryptase time course during peanut allergic reactions and establish a diagnostic cutoff to determine whether a food allergic reaction has taken place.

Previous studies have disputed the sensitivity of serum tryptase during a food allergic reaction stating that tryptase often fails to rise acutely. Indeed, using only the established upper bound (95th centile) of the tryptase assay to denote a rise in tryptase (>11.4 ng/mL), this statement is correct. Tryptase only rose above this level in 2.5% allergic reactions in our study. Previous studies on venom allergy have demonstrated that the sensitivity of the test can be improved if acute levels are compared with a baseline tryptase level taken after the reaction has resolved.^{7,8} This method detects rises that may occur within the normal range of the assay. When adopting this approach we observed a rise in tryptase compared with the baseline in 62.5% of all reactions and in 100% of anaphylactic reactions. Reactions in which tryptase failed to rise were distributed across all severity grades and were not necessarily the least severe reactions.

The amplitude of the tryptase rise is known to correspond with the allergic trigger. High peak tryptase levels are observed in allergic reactions involving anesthetics, intravenous drugs, and venom with smaller peaks observed in food allergic reactions.⁹ This is likely due to the former being associated with large doses of allergen being rapidly delivered directly into the systemic circulation with resultant extensive cutaneous and perivascular mast cell degranulation and mediator release. Hypotension is often a presenting feature of these reactions. This contrasts with food allergic reactions where allergen absorption occurs at a slower rate across the oropharyngeal and gastric mucosa and hypotension is rarely a feature¹⁰; thus the serum rises are likely to be much smaller. Minimal rises in tryptase may also reflect the limited extent of mast cell activation, for example, the gastrointestinal tract versus entire skin. Furthermore on release, mast cell tryptase may be secreted locally into the gut lumen in

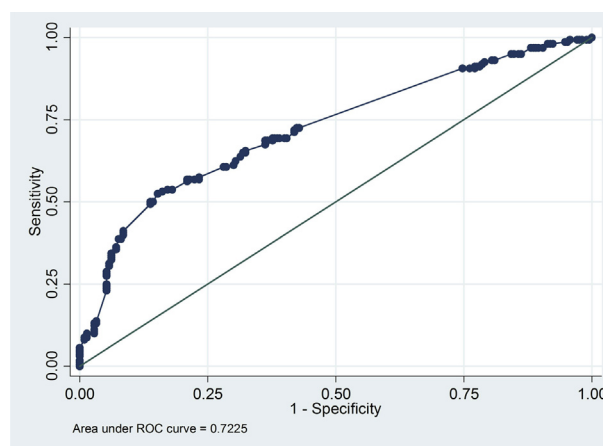


FIGURE 4. Receive operator characteristic (ROC) curve analysis to obtain the best value of ratio between peak tryptase during reaction and initial baseline serum tryptase to distinguish a reaction from a nonreaction.

predominantly gastrointestinal reactions or into laryngeal/bronchial secretions in dominantly respiratory reactions rather than the systemic circulation. Other possible explanations for this could be differences in mediator content of skin versus mucosal mast cells. Schwartz et al¹¹ reported that mucosal mast cells contained less tryptase compared with skin mast cells. Lastly it may be that changes in tryptase were too small to be detected by the assay, particularly if the patient had low starting levels of serum tryptase, for example, <1 ng/mL. It has previously been postulated that the failure to rise include a predominant basophil rather than mast cell involvement in an acute allergic reaction.³ Basophils are known to contain less than one hundredth of the amount of serum tryptase contained by mast cells.¹²

Other studies are often based on tryptase levels of patients presenting to emergency departments or postmortem samples and are therefore biased toward more severe reactions. Our study represents a wider spectrum of reactions including data on reactions of mild-to-moderate severity, 92.5% reactions in our sample as well as very severe reactions. We have shown, in line with previous studies, that the tryptase rise correlates with an increasing reaction severity.¹³ It has been shown previously that severe anaphylaxis is more likely to be associated with higher tryptase levels¹⁴ and peanut anaphylaxis is no exception. In our study, the mean rise in tryptase in the anaphylaxis group was 148.4% above the baseline level in contrast to 23.9% in the nonanaphylaxis group. Previous studies have attempted to relate individual symptoms to mediator release. Lin et al¹⁵ showed an association between the presence of tachycardia and urticaria and a rise in tryptase. Similarly in our study, we showed a significant relationship between the presence of dizziness and tachycardia and tryptase rise. Both of these are likely precursors to hypotension. We are the first to demonstrate that the presence of moderate-to-severe respiratory symptoms is associated with a higher tryptase. It follows that tryptase release most likely occurs mainly in the airways in respiratory predominant reactions. Regarding baseline tryptase as a predictor of reaction severity, Sahiner et al¹⁶ showed that serum baseline tryptase levels may predict moderate-to-severe anaphylaxis in children with food

TABLE III. Studies proposing optimal cutoffs for tryptase rise signifying mast cell activation or anaphylaxis

Author	Study design	Total number of patients	Criteria for tryptase increase	Criteria identify
Phadia AB, 2008 ²⁴	Observational study of nonallergic controls	126 healthy controls	Values above 11.4 ng/mL (upper 95th percentile)	Raised tryptase
Valent et al, 2012 ²⁵	Consensus review of published and unpublished data	Not stated	20% plus 2 ng/mL over baseline level	Mast cell activation
Enrique et al, 1999 ²⁶	Analysis of emergency admissions	17 adults with anaphylaxis	Levels >8.23 ng/mL	Anaphylaxis
Brown et al, 2009 ⁸	Prospective study of sting challenges	11 patients with anaphylaxis	Increase of 2 µg/L	Anaphylaxis
Egner et al, 2016 ²⁷	Retrospective study of perioperative anesthetic reactions	3400 patients with suspected reactions	Threshold increment of 20%	Mast cell mediator release
Wongkaewpothong et al, 2014 ²⁸	Prospective study of shrimp challenges	39 patients (12 anaphylaxis, 20 mild reactions, 7 controls)	Delta tryptase 0.8 µg/L	Anaphylaxis

allergy. We have not been able to prove a correlation between baseline tryptase levels and reaction severity. Our study, however, did not include any patients with raised baseline levels of tryptase, and other studies in venom allergic adults have established a clear relationship between elevated baseline levels of tryptase and severe anaphylaxis.¹⁷

Knowledge of tryptase kinetics is crucial in determining optimal sample timing to capture peak levels. Unlike other mediators such as histamine that are detectable within minutes of the onset of allergic symptoms, tryptase may not be detectable during the first 15 to 30 minutes.¹⁸ Mature tryptase is released in a complex heparin scaffold, thus slowing its release.¹⁹ Once in the circulation, the half-life is approximately 2 hours and levels are thought to diminish after this time. It is recommended therefore that the optimal time for sampling is between 30 minutes and 2 hours after the onset of a reaction. This recommendation was largely based on a hypothetical time course proposed by Schwartz²⁰ after his observations on 3 patients who underwent bee sting challenges and also 39 patients who developed anaphylaxis after a yellow jacket or honey bee sting. In this latter study by Van der Linden,¹ however, tryptase measurements were only taken up to 60 minutes with no further readings. Previously we have studied tryptase time course in anaphylaxis due to general anesthesia and found that peak tryptase levels in paired samples taken at 0 to 1 hour and 1 to 2 hours were statistically similar.²¹ In the current study, we also observed that peak levels did not occur immediately after the onset of reaction. However, in contrast with other studies where peaks have been observed in the majority of patients within 30 to 60 minutes of reaction onset,²² we noted that peak levels occurred later at 2 hours in the anaphylaxis group. One patient with severe anaphylaxis had a further reading taken in his recovery period at 3 hours that showed even higher levels compared with 2 hours. Unfortunately, no data on tryptase readings from 2 hours onwards were available in the remaining patients.

Limited data exist on the intraindividual variability of baseline tryptase over time. Generally, it is believed that serum tryptase remains stable. We showed that there was a variation of 14% in baseline tryptase readings over time within an individual. Some studies also suggest that there may be a diurnal variation in tryptase of up to 15%²³; however, we saw little variation in tryptase measurements taken over the course of the day during placebo challenges.

Having accounted for this inter-day variability we sought to establish a cutoff in tryptase rise above baseline to signify an allergic reaction. Currently, there is no consensus on the optimal cutoff for diagnosing either mast cell activation or anaphylaxis with different studies quoting different values (Table III). A cutoff point of 11.4 ng/mL is recommended by the commercial company Thermofisher for the ImmunoCAP assay based on this value being the 95th centile of tryptase levels in 126 nonallergic control subjects.²⁴ Valent et al²⁵ state that the acute serum total tryptase level should be an increase 20% plus 2 ng/mL over the baseline level of tryptase to be indicative of mast cell activation. Enrique et al²⁶ used absolute tryptase levels rather than a percentage rise to establish a rise of 8.23 ng/mL as a cutoff to identify anaphylaxis in patients. Other studies have defined a threshold difference of 2.0 ng/mL or more based on sting challenges during venom immunotherapy.⁸ Egner et al²⁷ studied tryptase during general

anesthesia reactions and established a threshold increment of 20% in tryptase to identify mast cell mediator release in an additional 14% of cases with peak tryptase between 5 and 14 ng/mL and a further 15% with levels below 5 ng/mL. Data on cutoffs specifically for food allergy are scarce. One study by Wongkaewpothong et al²⁸ identified a delta-tryptase cutoff of >0.8 $\mu\text{g/L}$ to confirm anaphylaxis in shrimp-induced reactions. In our study, we identify an optimal cutoff of a 30% rise in tryptase above baseline to signify an allergic reaction in peanut challenges.

Our study has some limitations. Patients were discharged when they had fully recovered and this was almost always within 2 hours of the onset of reaction. Therefore, we lack tryptase data on time points greater than 2 hours after the reaction. This is needed for a more complete time course and to corroborate exactly where peak levels are occurring. However, data on general anesthetic reactions show a substantial fall in tryptase from 2 to 4 hours, with the exception of those with extremely elevated peak tryptase levels of greater than 200 ng/mL.²¹ Moreover, it would have been useful to measure other allergic mediators such as histamine or platelet activating factor (PAF) to validate our tryptase findings with regard to mast cell activation. Furthermore, our data comprise reactions mainly of mild-to-moderate severity, and it is likely that more notable rises in tryptase would be observed if more severe reactions were included.

CONCLUSIONS

Acute tryptase measurement is of value in food allergic and normotensive reactions. There is a rise during reactions, but this is mostly within the normal range. It was raised above the normal range on only 4 of 160 reactions; thus, a single acute measurement of serum tryptase has poor sensitivity in the diagnosis of peanut allergic reactions. In food allergy, tryptase peaks are relatively low, and thus relating reaction levels to baseline levels is essential to capture a rise. We show that tryptase peaks may occur later at 2 hours in food-allergic reactions; therefore, readings both at initial and the 2-hour time points are important. We propose a cutoff in tryptase rise that may guide clinicians in establishing whether mast cell activation, and thus an IgE-mediated food allergic reaction, has occurred.

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TABLE E1. Four patients with acute tryptase readings above the normal range (>11.4 ng/mL)

Patient	Prechallenge tryptase (baseline) (ng/mL)	Peak tryptase during reaction (ng/mL)	Peak percentage rise (%)
1	4.5	12.2	271
2	6	17	283
3	4	13	325
4	3	12	400

All patients had severe anaphylaxis.