

Anaphylaxis and Clinical Utility of Real-World Measurement of Acute Serum Tryptase in UK Emergency Departments



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What is already known about this topic? Acute serum tryptase can be raised in anaphylaxis and current British guidelines recommend serial measurements. Sensitivity and specificity is published from the controlled environment of allergen challenge but not from the emergency department.

What does this article add to our knowledge? This British study is the first to document the sensitivity, specificity, and positive and negative predictive values of acute serum tryptase in emergency department anaphylaxis. Acute serum tryptase level of more than 12.4 ng/mL carries a high positive predictive value and specificity but poor sensitivity.

How does this study impact current management guidelines? Optimal real-world sampling of acute serum tryptase is difficult and acute serum tryptase is a poor biomarker for anaphylaxis. However, acute serum tryptase is useful in some situations to differentiate anaphylaxis from its mimics and should remain part of anaphylaxis assessment.

BACKGROUND: British guidelines recommend that serial acute serum tryptase measurements be checked in all adults and a subset of children presenting with anaphylaxis. This is the first study reporting the clinical utility of acute serum tryptase in a “real-world” emergency department (ED) setting following the publication of the World Allergy Organization (WAO) criteria for anaphylaxis.

OBJECTIVES: To (1) assess sensitivity, specificity, and positive and negative predictive values (PPV, NPV) of acute serum tryptase in anaphylaxis; (b) determine factors associated with

higher acute serum tryptase levels; and (c) audit compliance of acute serum tryptase measurement in the ED.

METHODS: The methods used were retrospective electronic search for ED admissions to 3 acute care hospitals in Birmingham, UK, with anaphylaxis in 2012 using wide search terms followed by scrutiny of electronic clinical records and application of the WAO diagnostic criteria for anaphylaxis. Patients with an acute serum tryptase measurement were included in the analysis.

RESULTS: Acute serum tryptase level was measured in 141 of 426 (33.1%) cases. Mean time from the onset of symptoms to the measurement of acute serum tryptase level was 4 hours 42 minutes (SD $\pm$ 05:03 hours) and no patients had serial measurements conforming to British guidelines. Acute serum tryptase level of more than 12.4 ng/mL (75th centile) was associated with a sensitivity, specificity, PPV, and NPV of 28%, 88%, 0.93, and 0.17, respectively. Multiple regression analysis showed that male sex (odds ratio, 2.66; $P = .003$) and hypotension (odds ratio, 7.08; $P = .001$) predicted higher acute serum tryptase level.

CONCLUSIONS: An acute serum tryptase level of more than 12.4 ng/mL in an ED setting carries high PPV and specificity, but poor sensitivity and NPV. © 2017 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2017;5:1280-7)

Key words: Anaphylaxis; Emergency department; Hypotension; Tryptase; ROC curve

Tryptase is a serine protease released from mast cells during an acute allergic reaction. Acute serum tryptase level measurement is advised in the evaluation of patients with anaphylaxis.^{1,2} British guidelines explicitly recommend that serial blood samples for

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Abbreviations used

ED- emergency department
NHS- National Health Service
NPV- negative predictive value
PPV- positive predictive value
ROC- receiver operator characteristic
WAO- World Allergy Organization

acute serum tryptase should be taken as soon as possible after the onset of symptoms, at 1 to 2 hours following symptom onset and a baseline sample at least 24 hours after the episode.¹ These timings reflect the half-life of tryptase, which is approximately 2 hours, with levels peaking 1 to 2 hours after onset and usually returning to baseline within 6 to 8 hours.^{3,4} The results are not immediately available to the emergency physician and therefore are not part of the initial evaluation but are used later in the follow-up of these patients at an allergy clinic.

The role for biomarkers of anaphylaxis, acute serum tryptase in particular, is unclear especially as seemingly severe episodes of anaphylaxis may not be associated with an elevated level^{3,5} and *vice versa*. Furthermore, there are currently no agreed international criteria for interpretation of acute serum tryptase in anaphylaxis with respect to a “cutoff” or a percentage change from a baseline measurement, although ≥ 11.4 ng/mL is frequently cited. Given that anaphylaxis in the community is nearly always an unpredictable event and is dealt with almost exclusively by emergency care providers, we felt it important to investigate acute serum tryptase in this group of patients. Previous studies investigating the utility of acute serum tryptase in an emergency department (ED) setting have involved prospective recruitment of patients with relatively small sample sizes, used different cutoff levels for interpretation, and were carried out before publication of the World Allergy Organization (WAO) diagnostic criteria for anaphylaxis, which now provide us with a unified, agreed definition of anaphylaxis. Moreover, they did not report sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).^{3,5-7} Our study is therefore an attempt to evaluate the performance of acute serum tryptase in a “real-world” setting.

We have recently published epidemiological data on 426 cases of anaphylaxis presenting to 3 EDs in Birmingham, United Kingdom, in 2012.⁸ Here, we present further data analysis of a subgroup of patients from the same cohort who had a measurement of acute serum tryptase when they attended the ED. The main objectives of the study were to (1) assess sensitivity, specificity, and PPV and NPV of acute serum tryptase in patients attending the ED; (2) determine factors associated with higher acute serum tryptase levels; and (3) audit compliance of acute serum tryptase measurement as per British guidelines.

METHODS

This is a retrospective, observational study of patients attending 3 busy EDs (total admissions in 2012: 251,215) with anaphylaxis/suspected anaphylaxis in one of the largest National Health Service (NHS) organizations in the United Kingdom (Heart of England NHS Foundation Trust). The catchment area of the 3 hospitals in the organization includes East and North Birmingham, Solihull, and South Staffordshire, a population of more than 890,000 people.

Patients

All ED attendances are coded using an “in-house” coding system. We carried out an electronic search of the database of ED attendances using a number of wide-reaching search terms to cover allergic presentations and retrieved 3516 potential cases of anaphylaxis. The search terms were as follows: “Allergic Reaction,” “Anaphylactic Shock,” “Anaphylaxis,” “Angioedema,” “Bite - Insect Non Venom,” “Bite - Insect Venom,” “Skin - Allergic Reaction,” “Skin - Rash, Other,” and “Skin - Urticaria.”

Next, 1 clinician scrutinized the scanned ED documentation, ambulance sheet, and other electronic information including clinic records where available. Through retrospective application of the WAO diagnostic criteria for anaphylaxis⁹ to patients’ presenting symptoms, cases of anaphylaxis were identified and data extracted. When possible, anaphylaxis mimics such as asthma and hereditary angioedema, among others, were excluded.

We also identified a small control group of patients (for constructing receiver-operating characteristic [ROC] curves) who presented to the ED with allergic symptoms in whom acute serum tryptase measurement was requested by the admitting physician, but did not fulfill the WAO diagnostic criteria for anaphylaxis.

To ensure the quality and reproducibility of the data extraction, initial data collection from a number of case notes was jointly performed by 2 clinicians to ensure appropriate inclusion and exclusion of cases and to improve consistency. Following this, when there was uncertainty from the first clinician about inclusion or exclusion of potential cases, these cases were jointly reviewed and discussed with the second clinician at weekly meetings during the data collection phase. When halfway through the data collection period, every previously assessed case was reassessed to eliminate bias associated with learned experience. In this reassessment, there were no cases that had been erroneously included; however, several had been missed and these were therefore incorporated into the data set.

Basic demographic data including age, sex, address, and ethnicity are available for every patient from the hospital database. Demographic data and data pertaining to symptoms (including time of onset) and comorbidities were extracted from the case records of every attendance that fulfilled the inclusion criteria using a standardized proforma and recorded in a spreadsheet (Microsoft Excel [2007]). Entries were coded and checked twice by 1 clinician. In addition, where patients had been reviewed by an allergy specialist, clinic records were analyzed to identify causative factors. As per the hospital policy, all cases of anaphylaxis and suspected anaphylaxis should be referred to the allergy clinic following discharge from the ED. An appointment is offered for allergy specialist review with 18 weeks from the date of referral (with an option of an urgent appointment in specific cases). Cases were assigned a severity grading according to the “Brown grading system.”¹⁰ Acute serum tryptase measurement, including the timing of samples, recorded on the electronic hospital database system, was extracted from the electronic system. Laboratory serum tryptase analysis was undertaken using a fluoroenzyme immunoassay (Immuno CAP-FEIA) on the ImmunoCAP platform (Phadia Thermo Fisher Scientific, Uppsala, Sweden).

Statistical analysis

Data were analyzed using SPSS (IBM Corp, Released 2013, IBM SPSS Statistics for Windows, Version 22.0, IBM Corp, Armonk, NY). Data were checked for outliers, skew, and kurtosis. Missing data were treated as “missing at random.” Four outliers were found and when removed skew and kurtosis were within acceptable levels for a normal distribution (<1.5). Data were analyzed with and without outliers and results were the same. Therefore, outliers were included in the data set to

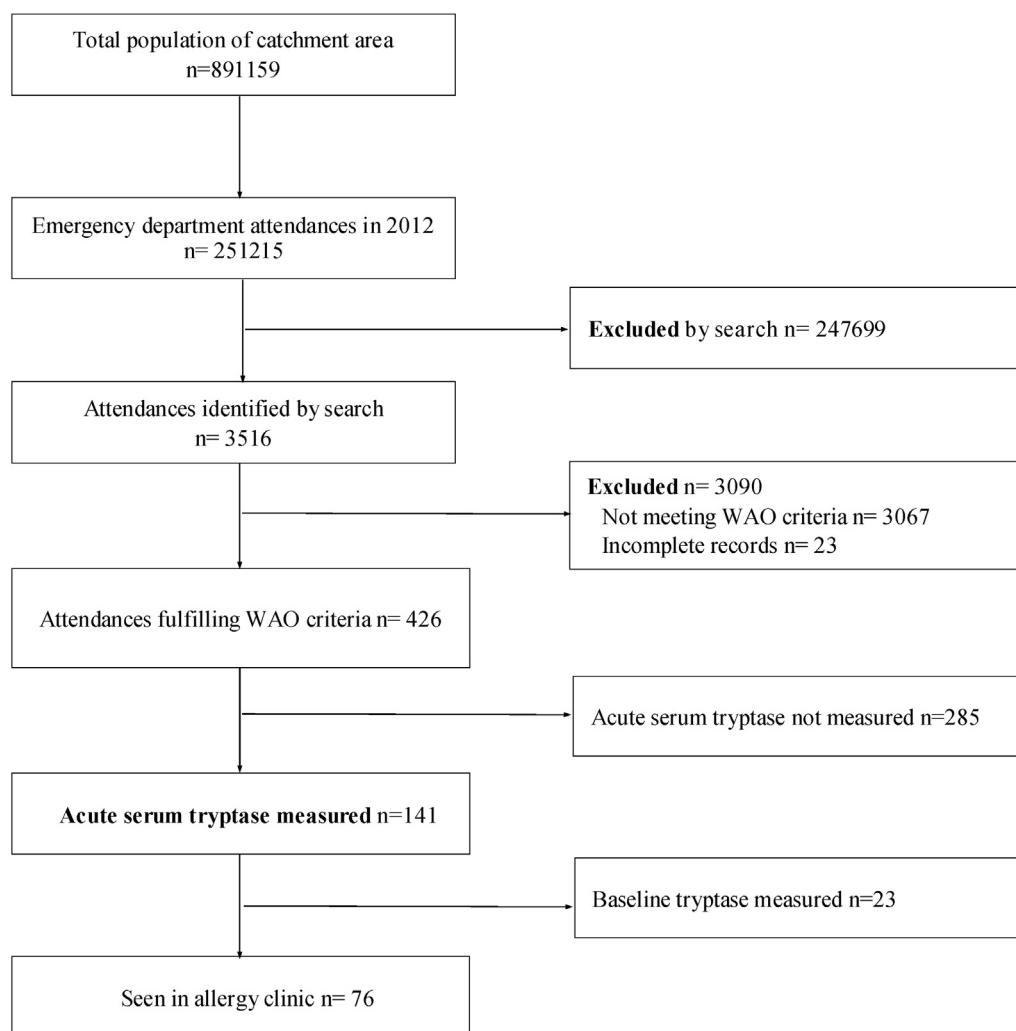


FIGURE 1. Study algorithm. A total of 3516 attendances to the ED during 2012 were identified using an electronic search of the attendances database using wide search terms. A total of 426 attendances fulfilled the WAO diagnostic criteria for anaphylaxis. A total of 141 patients had a measurement of acute serum tryptase, and 23 of these had a baseline tryptase measurement. Seventy-six were followed up in the allergy clinic.

ensure representativeness of the data. The relationships between acute serum tryptase and age and acute serum tryptase and time from symptom onset to sampling were analyzed using a Pearson correlation. Differences in mean acute serum tryptase levels across groups were analyzed using independent samples *t* tests. Where homogeneity of variance was significantly different (through inspection of the Levene test), the corrected “*t* value” and degrees of freedom (df) were used. Because of the large number of *t* tests run, alpha was set at 0.01. All tests were 2-tailed. A forced entry multiple regression model was run to investigate predictors of acute serum tryptase. All predictor variables that were significantly related to acute serum tryptase were included in the model. A logistic regression was also run to look at variables that might predict risk of high acute serum tryptase levels. Figures were constructed using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, Calif). The ROC curve was constructed from the acute serum tryptase measurements from the anaphylaxis group and non-anaphylaxis control group using Prism GraphPad 5.00. Sensitivity, specificity, PPVs, and NPVs were calculated at the 25th, 50th, and 75th centiles of acute serum tryptase measurements in the anaphylaxis group.

Data storage and institutional approval

To ensure data protection, data were stored on a physically and digitally secured computer at Birmingham Heartlands Hospital. The Microsoft Excel document was encrypted and all patient-identifiable information was removed before analysis. The project was reviewed by the Research and Development Department (in Heart of England NHS Foundation Trust) who verified that formal ethical approval was not required and then registered with the Clinical Governance Unit. The project required no external funding.

RESULTS

Study population

The study algorithm is summarized in [Figure 1](#). Of 426 attendances, 141 (33.1%) had an acute serum tryptase measurement. Demographic characteristics of the study sample are summarized in [Table I](#) and for the entire cohort in a recent publication by the authors along with extensive epidemiological analysis.⁸ In our cohort of 426 patients, 105 (24.6%) were children (<16 years) and of these children only 6 (5.7%) had a

TABLE I. Demographic characteristics of the study population

Characteristic	Acute serum tryptase level measured	Acute serum tryptase level not measured	Total	P value
Number (%) of cases of anaphylaxis	141 (33.1)	285 (66.9)	426	
Number (%) of severe cases*	56 (39.7)	89 (31.2)	145 (34.0)	.08
Sex, n (%)				
Male	55 (39.0)	116 (40.7)	171 (40.1)	.75
Female	86 (61.0)	169 (59.3)	255 (59.9)	
Age (y), mean $\pm$ SD	40.4 $\pm$ 20.2	26.7 $\pm$ 21.4	31.3 $\pm$ 22.0	<.001
Mean age of adults (those $\geq$ 16 y)	41.7 $\pm$ 19.5	38.4 $\pm$ 17.9	39.8 $\pm$ 18.7	.12
Patients with asthma, n (%)	43 (30.4)	78 (27.4)	121 (28.4)	.57

*Brown severity grading.¹⁰

measurement of acute serum tryptase. In children 86% of reactions were attributed to food and current British guidelines recommend acute serum tryptase measurement in children only in cases of drug-induced, venom-induced, or idiopathic anaphylaxis.⁸ The mean age of the group that had acute serum tryptase measured was higher than that of the group that did not.

Clinical manifestations

Forty-four of 141 (31.2%) patients had cardiovascular symptoms or signs, of which 15 (10.6%) had hypotension, 126 (89.3%) had respiratory involvement, with 41 (29.1%) having objective evidence of bronchospasm and 9 (6.4%) evidence of hypoxia. A total of 140 (99.3%) had skin or mucosal involvement, 42 (29.8%) had generalized urticaria, 87 (61.7%) had angioedema, and 35 (24.8%) had gastrointestinal involvement.

Acute serum tryptase

Mean acute serum tryptase level in the anaphylaxis group was 10.3 $\pm$ 10.4 ng/mL. In the control group of 25 patients, mean acute serum tryptase level was 6.8 $\pm$ 5.9 ng/mL (t = 1.65; df = 164; P = .10). In the anaphylaxis group, acute serum tryptase level was raised at or above 11.4 ng/mL (a previously accepted cutoff) in 46 (32.6%) cases. In the control group, 3 of 25 (12.0%) had an acute serum tryptase level of $\geq$ 11.4 ng/mL. A total of 132 of 141 (93.6%) had a recorded time of sampling on the electronic system. Of these, the mean time to the first acute serum tryptase level measurement from onset of symptoms was 4 hours and 42 minutes (SD $\pm$ 05:03 hours). There was a weak negative correlation between acute serum tryptase level and time between symptom onset and sampling (Pearson correlation coefficient, -0.19 ; P = .026) (see Figure E1 in this article's Online Repository at www.jaci-inpractice.org). There was no statistically significant difference in mean time of sampling in the severe and nonsevere anaphylaxis groups (t = 1.13; df = 133; P = .26) and no statistically significant difference in mean time of sampling across different etiologies. No patients had serial measurements of acute serum tryptase.

A total of 23 of 141 (16.3%) patients had a baseline serum tryptase more than 24 hours after symptom onset although none of these measurements was raised ($\geq$ 11.4 ng/mL). The median difference (delta) between acute serum tryptase and baseline tryptase was 115.9% (interquartile range, 26.5-238.2). Delta was greater than the previously quoted cutoff of 135% (11) for 9 of 23 (39.1%) cases. Thirteen of 45 (28.9%)

patients with an acute serum tryptase level of $\geq$ 11.4 ng/mL had a baseline tryptase measurement and 4 of 21 (19.0%) patients with acute serum tryptase level of $\geq$ 20 ng/mL had a baseline tryptase measurement. All the patients with baseline measurements were seen in the clinic. There was a statistically significant difference between mean baseline serum tryptase and acute serum tryptase in the group with severe anaphylaxis (n = 10; baseline 4.6 $\pm$ 1.3 ng/mL vs acute serum tryptase 19.6 $\pm$ 16.4 ng/mL; paired T test: t = 2.921; df = 9; P = .017), with a smaller, nonsignificant difference in the non-severe anaphylaxis group (n = 13; baseline 5.1 $\pm$ 2.0 ng/mL vs acute serum tryptase 9.4 $\pm$ 4.9 ng/mL; t = 2.032; df = 12; P = .065). Only 1 patient in the control group had a measure of baseline serum tryptase.

A mast cell disorder is considered in the differential diagnosis for all patients with spontaneous (idiopathic) anaphylaxis. Our clinical service considers a dermatology referral if cutaneous mastocytosis is suspected and the patient is also referred to a hematology clinic for consideration of bone marrow studies if the baseline serum tryptase level is $\geq$ 20 ng/mL. In the whole data set (those who presented to ED with anaphylaxis regardless of their attendance in the allergy clinic), 13 of 45 (28.9%) patients with an acute serum tryptase level of $\geq$ 11.4 ng/mL and 4 of 21 (19.0%) patients with an acute serum tryptase level of $\geq$ 20 ng/mL had a baseline tryptase measurement. Following completion of the study, we wrote to the family physicians of all those patients with a raised acute serum tryptase level ($>$ 11.4 ng/mL) and no record of baseline tryptase measurement to request that this be checked and that the report be brought to the attention of the allergy department if the baseline value is $\geq$ 11.4 ng/mL, so that a diagnosis of an underlying mast cell disorder could be considered. These additional measurements were not included in the study analysis.

Etiology of anaphylaxis

Seventy-six (53.9%) patients with an acute serum tryptase measurement were reviewed in person by an allergy specialist in the secondary care clinic (in Heart of England NHS Foundation Trust) for a comprehensive clinical evaluation and for further investigations as deemed necessary. All investigations were complete at the time of record review by the authors. Idiopathic anaphylaxis was the most common etiology (45 of 76 cases [59%]) and was twice as common in females as in males (35 of 49 [71.4%] vs 10 of 27 [37%], respectively; χ^2 = 7.16; df = 1; P = .008). There were

11 (14.5%) cases of food-induced anaphylaxis. In this group, mean acute serum tryptase level was 13.7 ± 10.6 ng/mL, with 6 of 11 (55.5%) patients having an acute serum tryptase level of ≥ 11.4 ng/mL and 3 of 11 (27.3%) having an acute serum tryptase level of ≥ 20 ng/mL. There were 15 (19.7%) cases of drug-induced anaphylaxis. In this group, mean acute serum tryptase level was 12.6 ± 15.2 ng/mL, with 5 of 15 (33.3%) patients having an acute serum tryptase level of ≥ 11.4 ng/mL and 2 of 15 (13.3%) having an acute serum tryptase level of ≥ 20 ng/mL. There were 45 (59.2%) cases of idiopathic anaphylaxis. In this group, mean acute serum tryptase level was 7.7 ± 8.3 ng/mL, with 8 of 45 (17.8%) having an acute serum tryptase level of ≥ 11.4 ng/mL and 4 of 45 (8.9%) having an acute serum tryptase level of ≥ 20 ng/mL. Idiopathic anaphylaxis was associated with lower acute serum tryptase levels than anaphylaxis due to drugs and food combined (mean, 7.6 ± 8.2 ng/mL vs 13.2 ± 13.1 ng/mL; $t = 2.26$; $df = 67$; $P = .027$). After thorough evaluation, no patient with idiopathic anaphylaxis was subsequently found to have indolent mastocytosis. There were no cases of venom-induced anaphylaxis in patients who had a measurement of acute serum tryptase, reflecting the low frequency of cases in our entire cohort of 426 cases. Further details regarding etiology of anaphylaxis in the entire cohort (regardless of acute serum tryptase measurement) can be found in our recent publication.⁸

Children

There were 6 children (<16 years old) who had a measurement of tryptase. Details are summarized in Table II.

ROC curve

The ROC curve (Figure 2) was generated using acute serum tryptase levels in patients presenting with anaphylaxis and acute serum tryptase levels in controls as described in the previous section. Data are summarized in quartiles of acute serum tryptase in Table III. Higher levels of acute serum tryptase (75th centile ≥ 12.4 ng/mL) showed high specificity (88.0%) and PPV (0.93) for anaphylaxis but showed poor sensitivity (27.8%) and NPV (0.17).

Factors associated with higher acute serum tryptase

Univariate analyses are summarized in Table E1 in this article's Online Repository at www.jaci-inpractice.org. Acute serum tryptase level was higher for patients with severe anaphylaxis as defined by the Brown grading system ($P = .006$), those with hypotension ($P = .012$), those with any cardiovascular symptoms ($P = .009$), those who had not self-administered an epinephrine autoinjector ($P = .002$), and males ($P = .001$). These were entered into a forced entry multiple regression model. The model was significant ($F = 8.98$ [5,140]; $P < .001$) and explained 25% of the variance ($R^2 = 0.25$; adjusted $R^2 = 0.22$). Hypotension (t value, -3.32 ; 95% CI, -15.59 to -3.94 ; $P = .001$) and male sex (t value, -3.05 ; 95% CI, -8.33 to -1.77 ; $P = .003$) emerged as significant predictors of a higher acute serum tryptase level (Table IV). A total of 42 of 141 (29.8%) patients had a history of asthma but this was not associated with acute serum tryptase (Table E1).

Predictors of high acute serum tryptase level

To see whether variables could be used to predict the likelihood of someone having a high or low acute serum tryptase level, a logistic regression model was constructed. We used a previously suggested acute serum tryptase level of

TABLE II. Children (<16 y old) with anaphylaxis and acute serum tryptase measurements*

Age	Sex	Severity (Brown grading)	Treated with epinephrine	Acute serum tryptase level (ng/mL)	Causative factor
5	Male	Severe	Yes	35.5	Nuts
6	Male	Severe	Yes	6.05	Milk
10	Female	Severe	Yes	3.82	Nuts
11	Female	Not severe	No	5.85	Cat
12	Male	Not severe	Yes	5.24	Nuts
12	Male	Not severe	Yes	12.1	Nuts
Mean: 9.3				Median: 5.95	

*Two of 6 (33.3%) children had an AT level of ≥ 11.4 ng/mL. None had a measurement of baseline tryptase available at the time of the study. None had hypotension or other evidence of cardiovascular compromise.

≥ 11.4 ng/mL as a cutoff between "raised" and "not raised" acute serum tryptase levels as the outcome variable. Variables that were significantly related to acute serum tryptase levels were entered into the model. This model was again significant ($\chi^2 = 29.66$; $P < .001$) and explained between 19% and 27% of the variance. Male sex and the presence of hypotension again significantly predicted increased odds of high acute serum tryptase levels (odds ratio 2.66 for male sex, 7.08 for hypotension; Table IV).

DISCUSSION

This is the first study to investigate the sensitivity and specificity of acute serum tryptase in cases of anaphylaxis presenting to the ED. It also offers an insight into the real-world ED scenario and how these conditions can influence the utility of such a test.

Anaphylaxis is a clinical diagnosis requiring prompt treatment and acute serum tryptase clearly does not constitute a part of the acute evaluation and management because results are not available to ED clinicians. Current British guidelines recommend 3 serial timed measurements of tryptase¹ and notably in our cohort this was not met in any patient. Although the relatively short timescale between the publication of these guidelines and our study could be cited as a potential reason for poor adherence, previous UK Resuscitation Council guidelines also recommended serial tryptase measurements in the same manner.¹¹ Multiple factors contribute to poor adherence: staff knowledge, a busy clinical environment, a "4-hour wait target" where 95% of patients must be reviewed, diagnosed, treated, and either discharged or admitted within 4 hours, and early discharge or transfer of patients. Acute serum tryptase results take several days to become available and thus are not available to ED clinicians who do not therefore see the benefits of the test. Many patients with milder cases of anaphylaxis do not require intravenous access or other blood tests and acute serum tryptase level measurement is therefore a low-priority task that adds to the workload of an already stretched team.

The time point of acute serum tryptase measurement (mean, 4 hours 42 minutes [SD $\pm$ 05:03 hours]) was such that the peak rise may have been missed in some cases. This however reflects a real-world clinical scenario because the timing of the acute serum tryptase sample depends on multiple factors including the rapidity of onset of symptoms, when the patient actually presents to the ED, how long it takes to stabilize the patient before the admitting

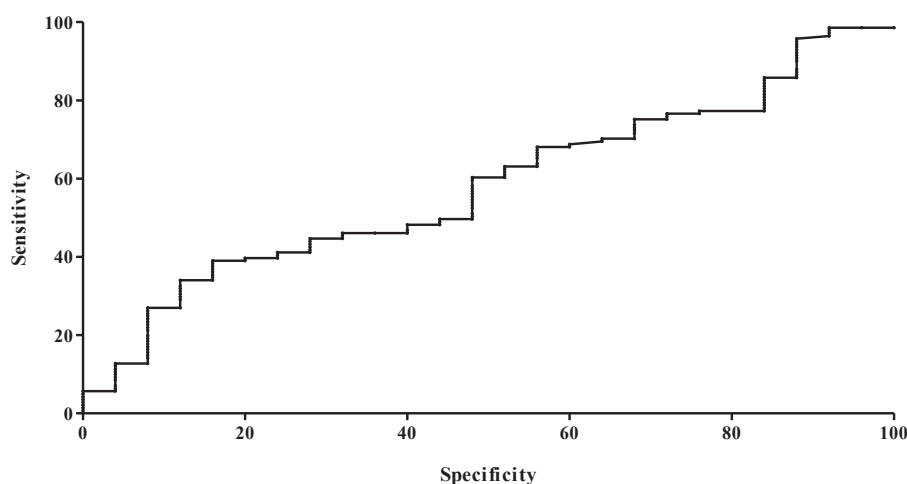


FIGURE 2. ROC curve showing acute serum tryptase level measurements in patients with anaphylaxis vs control group (patients not fulfilling the WAO diagnostic criteria for anaphylaxis) (area, 0.58; 95% CI, 0.47-0.69; $P = .19$). Table III shows sensitivity, specificity, NPV, and PPV for acute serum tryptase at different cutoffs.

TABLE III. Table associated with Figure 2 showing sensitivity, specificity, NPV, and PPV for acute serum tryptase at different cutoffs according to the ROC analysis

Acute serum tryptase level cutoff: 12.4 ng/mL						
75th centile	Anaphylaxis	Nonanaphylaxis	PPV	NPV	Sensitivity (%)	Specificity (%)
Tryptase level raised	38	3	0.93	0.17	27.8	88.0
Tryptase level not raised	104	22				
Acute serum tryptase level cutoff: 5.4 ng/mL						
50th centile	Anaphylaxis	Nonanaphylaxis	PPV	NPV	Sensitivity (%)	Specificity (%)
Tryptase level raised	72	9	0.89	0.19	50.7	64.0
Tryptase level not raised	70	16				
Acute serum tryptase level cutoff: 3.8 ng/mL						
25th centile	Anaphylaxis	Nonanaphylaxis	PPV	NPV	Sensitivity (%)	Specificity (%)
Tryptase level raised	107	18	0.86	0.17	75.4	28.0
Tryptase level not raised	35	7				

AT, Acute tryptase.

physician is able to obtain a sample for acute serum tryptase, and other confounding practical variables as stated above. However, Stone et al³ reported that although the acute serum tryptase peaks at 2 to 3 hours postexposure, levels can, in some patients, remain elevated at 50% above baseline for up to 10 hours.³ Previous studies have shown improved sensitivity of acute serum tryptase when serial measurements are taken¹²⁻¹⁴ but clearly in a busy clinical environment this may be challenging given the 4-hour wait target in the UK NHS as described above.

Wongkaewpothong et al¹⁴ and Braganza et al¹⁵ have reported considerably better sensitivity and specificity than our study at a lower acute serum tryptase level (50% and 85%, respectively, at 3.0 ng/mL and 55% and 93% at 9.0 ng/mL, respectively); however, these were in the context of allergen challenge or immunotherapy

where factors such as timing of the sample and definitive diagnosis of anaphylaxis can optimize the test. Previous ED-based studies of acute serum tryptase measurement have not addressed the sensitivity and specificity of the test.^{3,5-7,16} Here, we have for the first time constructed ROC curves and generated sensitivity, specificity, PPV, and NPV in a real-world ED setting. Using a cutoff of 12.4 ng/mL (75th centile), we show a sensitivity of 27.8%, specificity of 88%, and PPV and NPV of 0.93 and 0.17, respectively, indicating that acute serum tryptase measurement performs much more poorly in the ED environment. However, with a high specificity, when the acute serum tryptase level is raised, it is highly likely that the patient has experienced anaphylaxis. Previously, it has been reported that a rise of 135% or more (from baseline) in acute serum tryptase level is a better predictor of anaphylaxis than an absolute measurement

TABLE IV. Logistic regression analysis showing predictors of acute serum tryptase

Predictors	Standard error	Wald test	P value	Exp (B)	95% CIs	
					Lower	Upper
Sex (male)	.41	5.64	.02	2.66	1.19	5.95
Cardiovascular	.56	.70	.79	1.16	.39	3.44
Hypotension	.81	5.91	.01	7.08	1.46	34.33
Brown grading	.49	1.72	.19	.53	.20	1.37
Use of adrenaline autoinjector	1.16	1.90	.17	.20	.02	1.96

Cox and Snell $R^2 = 0.190$; Nagelkerke $R^2 = 0.265$; $-2 \log$ likelihood = 148.42. Statistically significant P values are given in bold.

alone.¹⁷ However, this was seen in only 39% of cases in our study. In our anaphylaxis cohort, only 23 of 141 (16.3%) patients had a baseline serum tryptase measurement (and only 1 in the control group), so we were unable to construct ROC curves for a percentage change from baseline. Only 28.9% of patients with an acute serum tryptase level of 11.4 ng/mL or more and 19.0% of patients with an acute serum tryptase level of 20 ng/mL or more had a baseline serum tryptase measurement. Mast cell disorders are considered for all patients with idiopathic anaphylaxis and are investigated and referred appropriately as described above.

We explored the factors that predict high acute serum tryptase levels using multiple and logistic regression models. A forced multiple regression model indicated that the presence of hypotension and male sex were the 2 factors that significantly predicted a higher acute serum tryptase level. This finding was supported with a logistic regression analysis showing that male sex and presence of hypotension significantly increase the probability of having levels of acute serum tryptase over the cutoff of ≥ 11.4 ng/mL. The association with hypotension is a finding that is in keeping with previously published data.^{5,6} However, the observation that patients with hypotension are 7 times more likely to have a raised acute serum tryptase has not been reported previously (Table IV). Thus, hypotension is a sensitive marker for an elevated acute serum tryptase level. The strong association of severe hypotension and higher acute serum tryptase is further supported by the high levels seen in patients developing severe cardiovascular perioperative anaphylaxis during general anesthesia, recently reported by our group¹⁸ and Mertes et al.¹⁹

The novel finding that male sex predicts higher acute serum tryptase warrants further investigation particularly because there is no known sex predisposition with respect to severity. Higher baseline serum tryptase in males has been reported previously,²⁰⁻²² which may at least in part explain this association. Also, rates of idiopathic anaphylaxis in our cohort were lower in males than in females (37.0% vs 71.4%) and idiopathic anaphylaxis was associated with lower acute serum tryptase levels. Previous studies have reported that food-induced anaphylaxis does not lead to an elevated acute serum tryptase level as often as other causes.^{7,23} However, in this study, we found that food-induced anaphylaxis leads to an elevated acute serum tryptase level as often as other causes. This may be related to relatively smaller sample size, wider CIs as well as the fact that a major comparison group is idiopathic anaphylaxis. Our finding that use of an epinephrine autoinjector is associated with lower acute serum tryptase level may highlight the importance of early administration of epinephrine in preventing a more severe systemic response to the allergen and thus preventing a rise in acute serum tryptase.

This study has limitations. First, we had a relatively small sample size, although the total number of cases in our cohort exceeds previous studies.^{3,5-7} In addition, only about a third of patients presenting with anaphylaxis had a measurement of acute serum tryptase, introducing selection bias although we have shown that among adults in our study, characteristics between groups are broadly similar but there is a trend toward more severe cases of anaphylaxis being included. The relatively small sample size is compounded by the low rates of requests for baseline tryptase measurement. Second, this was a retrospective study but all cases were carefully reviewed with respect to ED and ambulance crew observations and the sampling time for acute serum tryptase level was recorded accurately. Third, the control group that we used was small (25 patients). In the absence of a better control group, the use of this group of patients was a pragmatic choice, because patients with nonallergic conditions do not have routine acute serum tryptase level measurements. These patients had mucocutaneous findings *only* and therefore did not meet the WAO criteria for anaphylaxis, although it is plausible that treatment may have prevented some of them from developing anaphylaxis.

The role of acute serum tryptase level measurement in cases of suspected anaphylaxis remains debated despite being recommended in British and other national and international guidelines.^{1,2} Acute serum tryptase level measurement performs substantially better in the more controlled environments of allergen challenge and immunotherapy^{15,24} than it did in our study although an acute serum tryptase level of more than 12.4 ng/mL in ED patients does make the diagnosis of anaphylaxis highly likely. The high specificity of acute serum tryptase at this cutoff and its strong association with hypotension makes it a useful test in specific circumstances to distinguish anaphylaxis from its “mimics.” Also, it can be of use in patients presenting with isolated, severe hypotension (eg, insect-sting-induced anaphylaxis with hypotension alone, perioperative anaphylaxis, and Kounis syndrome) or in special groups such as the elderly, the visually impaired, those with a learning disability, or in cases in which there is paucity of historical or clinical information during assessment in an allergy clinic. Acute serum tryptase is of much less use in cases of sporadic anaphylaxis in otherwise normal patients where the clinical history yields the diagnosis. There is preliminary evidence supporting the role for newer biomarkers in anaphylaxis including chymase, carboxypeptidase, and dipeptyl peptidase 1.^{25,26} Further studies are required for investigating the clinical utility of these biomarkers in anaphylaxis.

In conclusion, our data have shown that acute serum tryptase has a high PPV and specificity, and low sensitivity and NPV in the diagnosis of anaphylaxis presenting to EDs of NHS hospitals in the United Kingdom. Hypotension and male sex were significant predictors of higher acute serum tryptase level and there is a suggestion that the use of an epinephrine autoinjector can prevent a rise in acute serum tryptase level. Furthermore, there was poor compliance with British guidelines with respect to serial acute serum tryptase level measurement in the 3 EDs included in this study. Serial acute serum tryptase level measurements may be impractical in the vast majority of cases of anaphylaxis that are seen, diagnosed, treated, and discharged from the ED within 4 hours.

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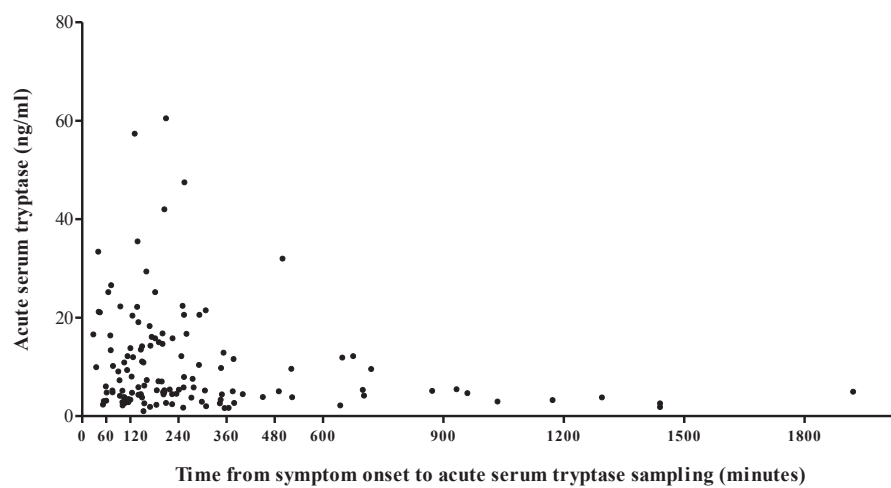


FIGURE E1. Correlation between acute serum tryptase level measurement and time from symptom onset to sampling. There is a weak negative correlation between acute serum tryptase level and time between symptom onset and sampling. Pearson correlation coefficient -0.19 ($P = .026$). $N = 132$ (9 did not have recorded timings).

TABLE E1. Univariate analysis: means $\pm$ SDs of acute serum tryptase levels across clinical characteristics

Clinical characteristic	N (Total = 141)	AT level, mean $\pm$ SD	t value (df)	P value
Sex				
Male	55	14.5 $\pm$ 13.2	3.53	.001
Female	86	7.7 $\pm$ 6.9	(73.26)	
Ethnicity				
White British	89	10.1 $\pm$ 9.6	-.45	.66
South Asian	39	11.0 $\pm$ 11.5	(126)	
Hypotension				
Yes	15	22.0 $\pm$ 17.3	2.87	.012
No	126	8.9 $\pm$ 7.6	(14.78)	
Cardiovascular symptoms				
Yes	44	14.5 $\pm$ 15.0	2.72	.009
No	97	8.4 $\pm$ 7.6	(54.85)	
Respiratory symptoms				
Yes	126	9.9 $\pm$ 9.8	-1.60	.12
No	15	14.3 $\pm$ 14.4	(139)	
Wheeze				
Yes	41	10.8 $\pm$ 11.2	.35	.96
No	100	10.1 $\pm$ 10.1	(139)	
Hypoxia				
Yes	9	10.5 $\pm$ 7.5	.05	.96
No	132	10.3 $\pm$ 10.6	(139)	
Stridor				
Yes	6	11.9 $\pm$ 12.9	.37	.71
No	135	10.3 $\pm$ 10.3	(139)	
Skin/mucosal involvement				
Yes	140	10.3 $\pm$ 10.4	NA	NA
No	1	10.9 $\pm$ 0		
Gastrointestinal symptoms		10.9 $\pm$ 10.2		
Yes	35	10.1 $\pm$ 10.5	.41	.69
No	106		(139)	
Brown grading				
Mild	85	8.1 $\pm$ 7.4	2.85	.006
Severe	56	13.6 $\pm$ 13.1	(78.17)	
Asthma				
Yes	42	8.8 $\pm$ 8.9	-1.13	.26
No	98	10.9 $\pm$ 11.0	(138)	
Use of angiotensin-converting enzyme inhibitors				
Yes	63	9.4 $\pm$ 6.6	-.29	.77
No	131	10.4 $\pm$ 10.6	(139)	
Use of beta blockers				
Yes	3	4.4 $\pm$ 1.3	-.99	.32
No	138	10.5 $\pm$ 10.5	(139)	
Used epinephrine autoinjector		4.7 $\pm$ 5.2		
Yes	12	10.8 $\pm$ 10.6	-3.47	.002
No	129	14.5 $\pm$ 13.2	(20.92)	
Administered epinephrine				
Yes	82	10.9 $\pm$ 11.6	.77	.45
No	59	9.5 $\pm$ 8.5	(139)	
Administered second epinephrine				
Yes	21	8.5 $\pm$ 8.5	-.85	.40
No	120	10.6 $\pm$ 10.7	(139)	
Needed intravenous fluid				
No	37	12.3 $\pm$ 12.2	1.36	.18
Yes	104	9.6 $\pm$ 9.6	(139)	

NA, Not applicable/available.

Statistically significant *P* values are given in bold.