Serum mast cell tryptase measurements: sensitivity and specificity for a diagnosis of anaphylaxis in emergency department patients with shock or hypoxaemia

Short title: Serum mast cell tryptase to diagnose anaphylaxis

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Abstract

Objective
Clinical diagnosis of anaphylaxis is principally based on symptoms and signs. However, particularly for patients with atypical symptoms, laboratory confirmation of anaphylaxis would be useful. This study investigated the utility of mast cell tryptase, an available clinical biomarker, for differentiating anaphylaxis from other causes of critical illness, which can also involve mast cell activation.

Methods
Tryptase was measured (ImmunoCAP) in serum from patients with anaphylaxis and non-anaphylactic critical illness (controls) at ED arrival, and after 1-2, 3-4, and 12-24 hours. Differences in both peak and delta (difference between highest and lowest) tryptase concentrations between groups were investigated using linear regression models, and diagnostic ability was analysed using Receiver Operating Characteristic curve analysis.

Results
Peak tryptase was 4.0-fold (95%CI: 2.9, 5.5) higher in anaphylaxis patients (n=67) than controls (n=120) (p<0.001). Delta-tryptase was 5.1-fold (95%CI: 2.9, 8.9) higher in anaphylaxis than controls (p<0.001). Optimal test characteristics (sensitivity: 72% (95%CI: 59, 82) and specificity: 72% (95%CI: 63, 80)) were observed when peak tryptase concentrations were >11.4ng/mL and/or delta-tryptase ≥2.0ng/mL. For hypotensive patients, peak tryptase >11.4ng/mL had improved test characteristics (sensitivity: 85% (95%CI: 65, 96) and specificity: 92% (95%CI: 85, 97)); the use of delta-tryptase reduced test specificity.

Conclusion
While peak and delta tryptase concentrations were higher in anaphylaxis than other forms of critical illness, the test lacks sufficient sensitivity and specificity. Therefore, mast cell tryptase
values alone cannot be used to establish the diagnosis of anaphylaxis in the ED. In particular, tryptase has limited utility for differentiating anaphylactic from non-anaphylactic shock.

**Key words:** Allergic shock, anaphylaxis, delta-MCT, emergency department diagnosis, and mast cell tryptase

**Introduction**

Anaphylaxis is a potentially life-threatening systemic allergic reaction involving multiple organ systems that progresses rapidly following exposure to an allergen.\(^1\)\(^-\)\(^3\) Diagnosis is straightforward in cases presenting with typical skin features (e.g. erythema, angioedema) together with objective evidence of additional organ system involvement (e.g. hypotension, hypoxaemia).\(^4\)\(^,\)\(^5\) However, cases can present with subtle skin changes that are easily missed or only appear after resuscitation from cardiovascular collapse.\(^6\)\(^,\)\(^7\) Furthermore, multisystem involvement may only be evident from subjective features (e.g. dyspnoea, nausea, dizziness) or potentially effort-dependent or psychogenic features (e.g. wheeze, stridor), which often resolve prior to first medical assessment.\(^1\)

Laboratory confirmation of anaphylaxis would be clinically useful for subsequent risk assessment and management. However, with the array of different mediators, multiple inflammatory pathways, and various cell types involved, no “gold standard” biomarker has been identified.\(^8\)\(^,\)\(^9\) The only widely available and well-standardised biomarker assay is a mast cell tryptase (MCT) test, measuring total tryptase in serum. Total tryptase is a combination of mature tryptases released following mast cell degranulation, which occurs following allergen exposure in most cases of anaphylaxis, plus baseline or “constitutive” pro-tryptases that are present regardless of anaphylactic state.\(^10\) Typically, serum MCT levels begin to rise within 30mins of symptom onset and peak after approximately 3hrs, before returning to baseline.\(^11\) The peak is
often missed when performing only a single measurement, or occurs within the normal range of the assay, minimising sensitivity. Furthermore, single measurements cannot differentiate anaphylaxis from clonal mast cell disorders that cause persistently high MCT values.\textsuperscript{12,13}

Serial measurements may overcome these issues by identifying peak concentrations and investigating changes in concentration (delta-MCT). Previously we have shown that an \textit{absolute} delta-MCT \( \geq 2\) ng/mL has a diagnostic sensitivity of 73\% and specificity of 98\% for anaphylaxis, compared to non-reacting, healthy controls.\textsuperscript{14} Another study investigating patients with a history of venom hypersensitivity (sting allergy), found that a \textit{relative} delta-MCT \( \geq 135\% \) was positive for 17/20 (85\%) patients who reacted upon re-exposure to venom and none of the 15 (0\%) non-reactors.\textsuperscript{15} These approaches have not been assessed for their ability to differentiate anaphylaxis from shock and/or hypoxaemia due to other causes, the most likely clinical scenario where an MCT assay would be measured.

The present study aimed to measure MCT concentrations in patients with shock and/or hypoxaemia from a range of causes, including anaphylaxis, and evaluate the diagnostic abilities of different methods for determining a positive result. We hypothesised that MCT release during non-anaphylactic shock would limit the diagnostic utility of MCT for differentiating anaphylactic from non-anaphylactic shock.

\textbf{Methods}

\textit{Patient recruitment}

Patients (\( \geq 16 \) yrs) meeting the inclusion criteria (Box 1) were enrolled in the Critical Illness and Shock Study (CISS)\textsuperscript{16} in the EDs of three Australian hospitals (Royal Perth Hospital, Perth WA; Armadale Kelmscott Memorial Hospital, Mount Nasura WA; and Austin Hospital, Heidelberg VIC) between March 2010 and January 2014. Clinical information and serum
samples were collected as soon as practicable after enrolment criteria were met, and after 1–2, 3–6, and 12–24 hours, where possible. Samples were stored immediately at -80°C.

*Ethics approval and consent*

Ethics approval was obtained (RPH and AKMH: EC 2009/080; AH: H2012/04477). As emergency care took priority, waiver of initial formal consent was approved under the provision of paragraph 2.3.6 of the National Health and Medical Research Council Ethical Conduct guidelines (2007). Patients could subsequently choose to provide delayed informed consent or withdraw from the study.

*Case selection*

Suspected anaphylaxis was identified on enrolment based on the National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network (NIAID/FAAN) consensus definition (Box 2). Atypical cases (e.g. ≥2 affected organ systems without skin reaction, or hypotension/wheeze where anaphylaxis was considered as a possible diagnosis) had no requirement for allergen exposure, as this was often uncertain at the time of presentation. Three clinical investigators (DF, GA, and SB), blinded to laboratory results, independently applied the NIAID/FAAN criteria, based on the available clinical information for each case. Cases were then classified as either definite (strict adherence to criteria) or possible anaphylaxis.

Sequentially enrolled non-anaphylaxis cases with hypotension (systolic BP <90mmHg) and/or hypoxaemia served as a control group.

*Mast cell tryptase measurement*

MCT concentrations in neat serum were determined using the clinical ImmunoCAP® Tryptase system (Phadia, Thermo Fisher Scientific, Uppsala, Sweden). In accordance with the product protocol, concentrations >11.4ng/mL were considered positive. The lower limit of detection was 0.707ng/mL.
**Statistical analysis**

Delta-MCT (difference between the highest and lowest values in each set of serial samples) were calculated as both an “absolute” concentration and a “relative” percentage change. Both peak MCT and absolute delta-MCT was log transformed to normalize the distribution of results. Differences between groups were investigated using linear regression models, adjusted for age and sex. To investigate the diagnostic utility of each method and investigate alternative cut-points, Receiver Operating Characteristic (ROC) curve analysis was used. The sensitivity, specificity, area under the (ROC) curve (AUC), and % correctly classified (%CC), with corresponding 95% confidence intervals (CIs) as appropriate, were calculated. Finally, we performed a descriptive analysis of confirmed anaphylaxis cases with negative MCT, and non-anaphylaxis cases with positive MCT (peak MCT >11.4ng/mL and/or absolute delta-MCT ≥2ng/mL) by comparing their main clinical features with those correctly classified, using linear regression models as appropriate. Statistical analyses were performed with Stata version 12.1 (StataCorp, College Station, Texas).

**Results**

**Patient characteristics**

Of the 83 patients enrolled with suspected anaphylaxis, 67 were classified as definite anaphylaxis (including all 36 severe cases), 15 as possible anaphylaxis, and 1 patient was given a diagnosis of chronic urticaria and excluded. The clinical characteristics of the definite and possible anaphylaxis cases are detailed in Table 1. Of the definite cases, moderate reactions were primarily triggered by foods (n=19/31(61%)) and severe reactions by drugs (n=20/36(56%)). Possible anaphylaxis reaction characteristics are described in Table S1. Possible anaphylaxis patients took 59 minutes (95%CI: 10, 109) longer from symptom onset to first blood sampling than definite anaphylaxis patients (p=0.020). No other differences were evident. The major
clinical characteristics of the non-anaphylaxis controls are described in Table S2. Control patients stayed in hospital 10.6 days (95%CI: 7.8, 13.5) longer than definite anaphylaxis patients (p<0.001), who in turn stayed for 7 hours (95%CI: 0.5, 14) longer than those with possible anaphylaxis (p=0.036).

Mast cell tryptase concentrations

Peak MCT concentrations, absolute delta-MCT concentrations, and relative delta-MCT percentages for definite anaphylaxis, possible anaphylaxis, and non-anaphylaxis controls are summarised in Figure 1. The median peak MCT concentration was 4.0-fold higher (95%CI: 2.9, 5.5) in definite anaphylaxis patients than controls (p<0.001), 2.6-fold higher (95%CI: 1.7, 3.9) in possible anaphylaxis than controls (p<0.001), and not different between definite and possible anaphylaxis patients (p=0.108). The median absolute delta-MCT of definite anaphylaxis patients was 5.1-fold higher (95%CI: 2.9, 8.9) than non-anaphylaxis controls (p<0.001). Possible anaphylaxis patients had a median absolute delta-MCT concentration 2.7-fold higher (95%CI: 1.1, 6.6) than controls (p=0.025). There was no difference between definite and possible anaphylaxis (p=0.123). Lastly, the median relative delta-MCT of definite anaphylaxis patients was 51% higher (95%CI: 14, 89) than that of controls (p=0.007). There were no differences between the other groups (p≥0.145).

Validation of current diagnostic limits

Using ROC curve analysis, the validity of currently recommended cut-points for peak MCT and absolute and relative delta-MCT14,15 (alone or in combination) to correctly diagnose anaphylaxis was investigated (Table 2, Figure 2). The combination of sensitivity, specificity, and %CC for peak or delta-MCT (absolute/relative) was not improved by selecting different cut-points. Major cut-points for peak MCT and absolute delta-MCT are listed in Tables S3 and S4 respectively, and the sensitivity, specificity, and %CC for each are detailed. The combined approach where a positive result was defined by a peak MCT >11.4ng/mL and/or absolute delta-
MCT $\geq 2$ng/mL optimized both the sensitivity and specificity, and correctly identified 72\%(95\%CI: 65, 78) of patients. When considering only patients (and controls) with hypotension, the diagnostic ability of MCT using all methods was improved (Table 2, Figure 3). In patients with hypotension, optimum sensitivity, specificity, AUC, and %CC was achieved when a positive result was defined by peak MCT alone.

**False positive and false negative MCT results**

The method describing a positive result as peak MCT $>11.4$ng/mL and/or *absolute* delta-MCT $\geq 2$ng/mL was investigated further to identify common features of outliers. Firstly, we considered the definite anaphylaxis cases with a negative MCT result (false negatives). These 19 patients spanned a large age range (18–69yrs), were primarily female (n=12/19 (63\%)), and stayed in hospital for 2–23hrs, all features of which were not significantly different from the correctly classified patients (p$\geq$0.070). The majority of these false negative patients had moderate reactions (n=15/19 (79\%) (p=0.001), and the major triggers were foods (n=12/19 (63\%)) and drugs (n=4/19 (21\%)). These patients displayed a variety of symptoms, and almost all were administered adrenaline (n=18/19 (95\%)), with 7/19 (39\%) of these receiving multiple doses. There were no common or unique features that defined this group. Their peak MCT ranged from 1.2–10.8ng/mL, and *absolute* delta-MCT ranged from 0.1–1.9ng/mL.

Lastly, we considered the non-anaphylaxis controls with a positive peak MCT and/or *absolute* delta-MCT result (false positives). Thirty-four such patients were identified, being between 22 and 86 years, primarily male (n=25/34 (74\%)), and stayed in hospital for 0–50 days. These features were not significantly different from controls with negative MCT results (p$\geq$0.094). All of these patients had hypotension, and the predominant diagnoses were sepsis (n=12/34 (35\%)), cardiac conditions (n=8/34 (24\%)), and trauma (n=7/34 (21\%)). Their peak MCT ranged from 2.8–44.3ng/mL, and *absolute* delta-MCT ranged from 1.9–22.3ng/mL.
Discussion

Laboratory confirmation of a diagnosis of anaphylaxis is important to facilitate risk minimization strategies (trigger avoidance, desensitization therapy, and access to adrenaline auto-injectors (e.g. EpiPen®)) for susceptible patients. Serial MCT measurements are moderately effective at differentiating anaphylactic patients from healthy/non-reacting individuals. This study is the first to investigate MCT as a diagnostic marker, to differentiate anaphylaxis from other types of shock in the emergency setting.

Both peak and absolute delta-MCT concentrations were higher in patients with anaphylaxis compared to critically ill non-anaphylactic controls. However, not all anaphylactic patients had disturbances in MCT and, conversely, several non-anaphylactic controls had abnormal MCT. Indeed, other studies have also shown MCT is not elevated in a considerable proportion of anaphylaxis cases. The best test characteristics we found used a combined approach defining a positive result as a peak MCT >11.4ng/mL and/or an absolute delta-MCT ≥2.0ng/mL. This provided better sensitivity, but specificity was still poor. When the analysis focused on hypotensive patients (excludes moderate anaphylaxis), the sensitivity of peak MCT in particular was greatly improved, without compromising specificity. We have previously shown that MCT concentrations correlated with hypotension in anaphylaxis patients, so the increased sensitivity was predictable, if not obvious. However, the finding that peak MCT retained a high specificity for anaphylaxis amongst other critical illnesses with associated hypotension, is novel. Hypotension was frequently observed in non-anaphylactic controls without detectable changes in MCT, and it is therefore unlikely that hypotension alone triggers sufficient mast cell activation to alter systemic MCT concentrations. When considered in combination with clinical features, particularly hypotension, elevated/altered serum MCT concentrations can provide useful additional information to assist with the diagnosis of anaphylaxis. However, a low or negative MCT should not be used to exclude anaphylaxis.
Disturbances in MCT concentrations are not solely attributed to allergy and anaphylaxis. In addition to clonal mast cell disorders like mastocytosis, which are associated with elevated baseline MCT levels due to abnormally high numbers of mast cells, elevated MCT has previously been observed in non-anaphylactic deaths and trauma patients due to cell lysis. In this study, we also observed elevated MCT levels in trauma patients. This is most likely the result of the physical stresses of trauma causing mast cell damage and release of mediators, including MCT. Furthermore, it is also possible that delta-MCT measurements may not accurately reflect mast cell activation in patients who receive large volumes of fluid therapy; significant haemodilution may cause a positive delta-MCT (decrease from pre- to post-treatment) despite a lack of substantial mast cell involvement.

Not all cases of anaphylaxis involve mast cell activation. There are a number of other mechanisms of anaphylaxis that have been proposed, including non-immunological pathways, with varying degrees of evidence in humans. A lack of mast cell involvement would account for a normal and stable serum MCT concentration in some patients. Due to the large degree of variation in symptoms, severity, triggers, routes of allergen exposure, and immune mechanisms, it seems unlikely that any single biomarker test will have sufficient sensitivity and specificity to establish a diagnosis of anaphylaxis, particularly in cases where the physiological shock may be attributable to, or complicated by, another cause.

Limitations

We acknowledge that the delta-MCT values obtained in this study may not fully represent the change due to illness, as the design of the CISS protocol sampled patients only until discharge. This is in contrast to other studies that measured baseline MCT weeks after the event. However, the purpose of our study was to identify events measurable during ED presentation and it was important to determine whether the delta-MCT concentration
measureable during this period could be useful diagnostically. As there is no ‘gold standard’ test for anaphylaxis, the ROC curve analysis was made difficult. We used the current, widely accepted NIAID/FAAN criteria for diagnosing true cases, with the diagnosis made by experienced emergency physicians blinded from the laboratory results. As these criteria are still open to interpretation, we minimized potential bias by having multiple clinicians independently apply the criteria to each case.

Conclusion

In conclusion, although both median peak and delta-MCT concentrations are higher in anaphylaxis than other forms of critical illness, the sensitivity and specificity of MCT is not useful as a diagnostic tool. Setting a cut-point for either peak or delta-MCT to determine a dichotomous positive/negative result cannot be used to establish the diagnosis of anaphylaxis in the ED. Whilst MCT is somewhat helpful at identifying acute anaphylaxis when compared to healthy/non-reacting individuals, it has limited utility for differentiating anaphylactic from non-anaphylactic shock. Therefore, when a clinical judgment of anaphylaxis is made, referral to an allergy specialist is required, regardless of the MCT result.

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Disclosure
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*Competing interests*

None declared.
References


**Box 1: Critical Illness and Shock Study (CISS) inclusion criteria**

1. **Shock** – ANY cause (e.g. septic, traumatic, haemorrhagic, cardiogenic, anaphylactic), with any of:
   a. systolic BP < 90 mmHg, or
   b. mean arterial pressure (MAP) ≤ 65 mmHg, or
   c. heart rate (HR) ≥ systolic BP (i.e. shock index HR/systolic BP ≥ 1), or
   d. lactate ≥ 4 mmol/L

2. **Hypoxaemic respiratory failure** – ANY cause (e.g. infective, traumatic, cardiogenic, non-cardiogenic), with either:
   a. keeping SpO₂ > 90% requiring > 6 L/min O₂ by face mask, or
   b. PaO₂ (mmHg)/FiO₂ < 200 (Ventilated, BiPAP, Venturi mask)

3. **Post-cardiac arrest**

4. **Severe sepsis**, defined as: sepsis (likely infection +2 or more of; temp > 38°C or < 36°C, HR > 90, respiratory rate > 20, white cell count > 12 or < 4) plus either:
   a. shock or hypoxaemic respiratory failure as defined above, or
   b. organ dysfunction (oliguria/acute renal failure or acute altered mental state)

5. **Acute anaphylaxis** defined as a reaction, which at the time of enrolment involves two or more organ systems:
   a. Skin: generalized erythema or itch, urticaria, angioedema
   b. Gastrointestinal tract: nausea, vomiting, abdominal or pelvic pain, incontinence
   c. Respiratory system: dyspnoea, wheeze, stridor, chest tightness, hypoxaemia (SpO₂ ≤ 92%)
   d. Cardiovascular system: diaphoresis, dizziness/pre-syncope, collapse, altered mental state, hypotension (systolic BP < 90/MAP < 65, or relative BP drop of > 30% from normal for that individual)

OR:

Any *acute onset* (minutes-hours) of *hypotension* or *bronchospasm* where anaphylaxis is considered possible, even if the typical skin features listed above are not present.
Box 2: NIAID/FAAN clinical criteria for diagnosing anaphylaxis

**Anaphylaxis is highly likely when any one of the following 3 criteria are fulfilled:**

1. Acute onset of an illness (minutes to several hours) with involvement of the skin, mucosal tissue, or both (e.g., generalized hives, pruritus or flushing, swollen lips-tongue-uvula)

   **AND AT LEAST ONE OF THE FOLLOWING:**
   
   a. Respiratory compromise (e.g., dyspnoea, wheeze-bronchospasm, stridor, reduced PEF, hypoxaemia)
   
   b. Reduced BP or associated symptoms of end-organ dysfunction (e.g., hypotonia [collapse], syncope, incontinence)

2. Two or more of the following that occur rapidly after exposure to a likely allergen† for that patient (minutes to several hours):

   a. Involvement of the skin-mucosal tissue (e.g., generalized hives, itch-flush, swollen lips-tongue-uvula)
   
   b. Respiratory compromise (e.g., dyspnoea, wheeze-bronchospasm, stridor, reduced PEF, hypoxaemia)
   
   c. Reduced BP or associated symptoms (e.g., hypotonia [collapse], syncope, incontinence)
   
   d. Persistent gastrointestinal symptoms (e.g., crampy abdominal pain, vomiting)

3. Reduced BP after exposure to known allergen† for that patient (minutes to several hours):

   a. Infants and children: low systolic BP (age specific) or greater than 30% decrease in systolic BP‡
   
   b. Adults: systolic BP of less than 90 mmHg or greater than 30% decrease from that person’s baseline

†For the purposes of Definitions 2 and 3 we considered that the conditions for allergen exposure were met if the patient had been exposed to either (i) an infrequently encountered precipitant just prior to onset, such that both exposure and the occurrence of anaphylaxis were unlikely to be coincidental or (ii) a commonly encountered substance (e.g. food) if it was a previously known allergen for that patient or subsequently identified through allergen-specific IgE testing and/or challenge testing to be the likely cause. ‡Not applicable in this study as all participants were aged 16 years or older.

NIAID/FAAN, National Institute of Allergy and Infectious Diseases/Food Allergy and Anaphylaxis Network; PEF, peak expiratory flow.
### TABLE 1: Clinical reaction features of possible and definite anaphylaxis presentations

<table>
<thead>
<tr>
<th></th>
<th>Possible anaphylaxis</th>
<th>Definite anaphylaxis</th>
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<tbody>
<tr>
<td><strong>n</strong></td>
<td>15</td>
<td>67</td>
</tr>
<tr>
<td><strong>Age (years), mean (SD)</strong></td>
<td>45 (18)</td>
<td>40 (16)</td>
</tr>
<tr>
<td><strong>Male gender, n (%)</strong></td>
<td>8 (53)</td>
<td>30 (45)</td>
</tr>
<tr>
<td><strong>Suspected cause†</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food, n (%)</td>
<td>6 (40)</td>
<td>22 (33)</td>
</tr>
<tr>
<td>Summative (food + exercise), n (%)</td>
<td>–</td>
<td>3 (4)</td>
</tr>
<tr>
<td>Injected diagnostic contrast, n (%)</td>
<td>–</td>
<td>2 (3)</td>
</tr>
<tr>
<td>Injected medicine, n (%)</td>
<td>–</td>
<td>2 (3)</td>
</tr>
<tr>
<td>Oral medicine, n (%)</td>
<td>3 (20)</td>
<td>23 (34)</td>
</tr>
<tr>
<td>Venoms, n (%)</td>
<td>3 (20)</td>
<td>5 (7)</td>
</tr>
<tr>
<td>Unknown or other, n (%)</td>
<td>3 (20)</td>
<td>10 (15)</td>
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<tr>
<td><strong>Onset to enrolment (mins), median (IQR)</strong></td>
<td>125 (140)</td>
<td>75 (50)</td>
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<tr>
<td><strong>Any skin feature, n (%)</strong></td>
<td>14 (93)</td>
<td>65 (97)</td>
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<tr>
<td>Urticaria, n (%)</td>
<td>7 (47)</td>
<td>40 (60)</td>
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<tr>
<td>Erythema, n (%)</td>
<td>12 (80)</td>
<td>47 (70)</td>
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<tr>
<td>Oedema, n (%)</td>
<td>4 (27)</td>
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<tr>
<td>Periorbital oedema, n (%)</td>
<td>2 (13)</td>
<td>18 (27)</td>
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<tr>
<td><strong>Any gastrointestinal feature, n (%)</strong></td>
<td>10 (67)</td>
<td>29 (43)</td>
</tr>
<tr>
<td>Nausea</td>
<td>9 (60)</td>
<td>27 (40)</td>
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<tr>
<td>Vomiting</td>
<td>3 (20)</td>
<td>10 (15)</td>
</tr>
<tr>
<td>Abdominal/pelvic pain</td>
<td>–</td>
<td>8 (12)</td>
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<tr>
<td><strong>Any respiratory feature, n (%)</strong></td>
<td>12 (80)</td>
<td>59 (88)</td>
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<tr>
<td>Chest/throat tightness</td>
<td>12 (80)</td>
<td>41 (61)</td>
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<td>Dyspnoea, n (%)</td>
<td>2 (13)</td>
<td>39 (58)</td>
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<td>Stridor, n (%)</td>
<td>1 (7)</td>
<td>6 (9)</td>
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<tr>
<td>Wheeze, n (%)</td>
<td>2 (13)</td>
<td>25 (37)</td>
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<td>Hypoxaemia, n (%)</td>
<td>–</td>
<td>9 (13)</td>
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<tr>
<td><strong>Any cardiovascular feature, n (%)</strong></td>
<td>7 (47)</td>
<td>42 (63)</td>
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<tr>
<td>Dizziness</td>
<td>6 (40)</td>
<td>19 (28)</td>
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<tr>
<td>Diaphoresis</td>
<td>2 (13)</td>
<td>20 (30)</td>
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<td>Hypotension (systolic BP &lt; 90 mmHg), n (%)</td>
<td>–</td>
<td>26 (39)</td>
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<tr>
<td>Incontinence</td>
<td>–</td>
<td>3 (4)</td>
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<tr>
<td>Confusion</td>
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<td>8 (12)</td>
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<tr>
<td>Collapse</td>
<td>–</td>
<td>10 (15)</td>
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<tr>
<td>Loss of consciousness</td>
<td>–</td>
<td>6 (9)</td>
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<tr>
<td><strong>Treated with adrenaline, n (%)</strong></td>
<td>13 (87)</td>
<td>63 (94)</td>
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<td>Multiple doses, n (%)</td>
<td>2 (13)</td>
<td>28 (42)</td>
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<tr>
<td><strong>Length of stay (hrs), median (IQR)</strong></td>
<td>5 (3)</td>
<td>7 (11)</td>
</tr>
<tr>
<td><strong>Death, n (%)</strong></td>
<td>–</td>
<td>–</td>
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</tbody>
</table>

†The most common medications responsible for anaphylactic episodes were antibiotics (14 (47%)), followed by NSAIDs (10 (33%)), intravenous contrast (2 (7%)), and vaccines (2 (7%)).
The most common foods implicated were nuts and legumes (15 (48%)), followed by seafood (3 (10%)), fruits and vegetables (3 (10%)), and meat, egg and dairy (1 (3%)). The most common venom trigger was honeybee (7 (88%)), followed by paper wasp (1 (12%)).

SD, standard deviation; IQR, interquartile range.
TABLE 2: Sensitivity, specificity, AUC, and %CC for the diagnosis of anaphylaxis using different methods†

<table>
<thead>
<tr>
<th>Method</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>AUC</th>
<th>%CC</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak MCT &gt;11.4ng/mL</td>
<td>58.2 (45.5, 70.2)</td>
<td>93.3 (87.3, 97.1)</td>
<td>0.76 (0.69, 0.82)</td>
<td>81</td>
</tr>
<tr>
<td>Absolute delta-MCT ≥2ng/mL</td>
<td>67.2 (54.6, 78.2)</td>
<td>72.5 (63.6, 80.3)</td>
<td>0.70 (0.63, 0.77)</td>
<td>71</td>
</tr>
<tr>
<td>Peak MCT &gt;11.4ng/mL and/or delta-MCT ≥2ng/mL</td>
<td>71.6 (59.3, 82.0)</td>
<td>71.7 (62.7, 79.5)</td>
<td>0.72 (0.65, 0.78)</td>
<td>72</td>
</tr>
<tr>
<td>Peak MCT &gt;11.4ng/mL and/or delta-MCT ≥7ng/mL</td>
<td>58.2 (45.5, 70.2)</td>
<td>91.7 (85.2, 95.9)</td>
<td>0.75 (0.68, 0.81)</td>
<td>80</td>
</tr>
<tr>
<td>Relative delta-MCT ≥135%</td>
<td>59.7 (47.0, 71.5)</td>
<td>52.5 (43.2, 61.7)</td>
<td>0.56 (0.49, 0.64)</td>
<td>55</td>
</tr>
<tr>
<td>Hypotensive patients only§ (systolic BP &lt; 90mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak MCT &gt;11.4ng/mL</td>
<td>84.6 (65.1, 95.6)</td>
<td>92.2 (85.3, 96.6)</td>
<td>0.88 (0.81, 0.96)</td>
<td>91</td>
</tr>
<tr>
<td>Absolute delta-MCT ≥2ng/mL</td>
<td>88.5 (69.8, 97.6)</td>
<td>69.9 (60.1, 78.5)</td>
<td>0.79 (0.71, 0.87)</td>
<td>74</td>
</tr>
<tr>
<td>Peak MCT &gt;11.4ng/mL and/or delta-MCT ≥2ng/mL</td>
<td>96.2 (80.4, 99.9)</td>
<td>68.9 (59.1, 77.7)</td>
<td>0.83 (0.77, 0.88)</td>
<td>74</td>
</tr>
<tr>
<td>Peak MCT &gt;11.4ng/mL and/or delta-MCT ≥7ng/mL</td>
<td>84.6 (65.1, 95.6)</td>
<td>91.3 (84.1, 95.9)</td>
<td>0.88 (0.80, 0.96)</td>
<td>90</td>
</tr>
<tr>
<td>Relative delta-MCT ≥135%</td>
<td>65.4 (44.3, 82.8)</td>
<td>51.5 (41.4, 61.4)</td>
<td>0.58 (0.48, 0.69)</td>
<td>56</td>
</tr>
<tr>
<td>Peak MCT &gt;11.4ng/mL and/or delta-MCT ≥135%</td>
<td>92.3 (74.9, 99.1)</td>
<td>48.5 (38.6, 58.6)</td>
<td>0.70 (0.63, 0.78)</td>
<td>57</td>
</tr>
</tbody>
</table>

†Values were determined using ROC curve analysis and are displayed as the estimate (95% CI), or % only for CC. ‡All patients included all definite anaphylaxis cases (n=67) and all non-anaphylaxis controls (n=120). §Hypotensive patients only included the definite anaphylaxis cases with hypotension (n=26) and non-anaphylaxis controls with hypotension (n=103).

AUC, area under the (ROC) curve; CC, correctly classified; MCT, mast cell tryptase; ROC, Receiver Operating Characteristic.
Figure Legends

Figure 1: Summary of the mast cell tryptase (MCT) concentrations measured in singlicate in neat sera using diagnostic, validated ImmunoCAP methodology. Samples were collected from critically ill non-anaphylaxis controls (e.g. sepsis, trauma) (n=120), possible (n=15) and definite (n=67) anaphylaxis patients over the first 24 hours since ED admission. Definite anaphylaxis patients strictly adhered to the NIAID/FAAN criteria for diagnosis, whilst possible anaphylaxis patients did not meet criteria but their most likely diagnosis was anaphylaxis. Boxplots identify the median, upper and lower quartiles, and the range. A) Peak MCT concentrations. Dashed line indicates a peak MCT of 11.4ng/mL, the 95th percentile of healthy controls. B) Absolute delta-MCT concentrations (change in concentration between the highest and lowest values). Dashed line indicates an absolute delta-MCT of 2.0ng/mL. C) Relative delta-MCT percentages (percentage change between the highest and lowest values). Dashed line indicates a relative delta-MCT of 135%.

Note: p-values are derived using linear regression models testing the ratio of the two means following log transformation, adjusted for age and sex.

Figure 2: Receiver Operating Characteristic (ROC) curves showing sensitivity and specificity of A) peak mast cell tryptase (MCT); B) absolute delta-MCT; and C) relative delta-MCT for a diagnosis of anaphylaxis in a sample of 67 anaphylaxis cases (defined using NIAID/FAAN criteria) and 120 non-anaphylaxis critically ill controls, with ng/mL (A and B) or % (C) cut-offs indicated. MCT was measured in singlicate using the diagnostic, validated ImmunoCAP protocol in neat serum samples collected from patients over the first 24 hours since their enrolment to the ED.
Figure 3: Receiver Operating Characteristic (ROC) curves showing sensitivity and specificity of A) peak mast cell tryptase (MCT); B) absolute delta-MCT; and C) relative delta-MCT for a diagnosis of anaphylaxis in a sample of 26 anaphylaxis cases (defined using NIAID/FAAN criteria) with hypotension (systolic BP <90mmHg) and 103 non-anaphylaxis critically ill controls with hypotension, with ng/mL (A and B) or % (C) cut-offs indicated. MCT was measured in singlicate using the diagnostic, validated ImmunoCAP protocol in neat serum samples collected from patients over the first 24 hours since their enrolment to the ED.