

# Diagnostic Value of Histamine and Tryptase Concentrations in Severe Anaphylaxis with Shock or Cardiac Arrest during Anesthesia

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## ABSTRACT

**Background:** The diagnosis of acute life-threatening allergic reactions during anesthesia relies on clinical signs, histamine and/or tryptase measurements, and allergic testing. In patients who die after the reaction, skin tests cannot be performed, and the effect of resuscitation manoeuvres on mediator concentrations is unknown. The authors compared plasma histamine and tryptase concentrations in patients with severe allergic reactions during anesthesia with those measured in patients with shock due to other causes.

**Methods:** Patients with life-threatening allergic reactions were retrieved from a previous database (Group ALLERGY). All had positive allergy tests to administered agents. Patients with severe septic/cardiogenic shock or cardiac arrest (Group CONTROL) had histamine and tryptase measurements during resuscitation manoeuvres. Receiver operating characteristics curves were built to calculate the optimal mediator thresholds differentiating allergic reactions from others.

**Results:** One hundred patients were included, 75 in Group ALLERGY (cardiovascular collapse, 67; cardiac arrest, 8) and 25 in Group CONTROL (shock, 11; cardiac arrest, 14). Mean histamine and tryptase concentrations remained unchanged throughout resuscitation in Group CONTROL and were significantly higher in Group ALLERGY. The optimal thresholds indicating an allergic mechanism were determined as 6.35 nmol/l for histamine (sensitivity: 90.7% [95% CI, 81.7 to 96.1]; specificity: 91.7% [73.0 to 98.9]) and 7.35 µg/l for tryptase (sensitivity: 92% [83.4 to 97.0]; specificity: 92% [73.9 to 99.0]).

**Conclusions:** Resuscitation manoeuvres by themselves did not modify mediator concentrations. Virtually all life-threatening reactions during anesthesia associated with mediator concentrations exceeding the thresholds were allergic events. These findings have potential forensic interest when a patient dies during anesthesia. (*ANESTHESIOLOGY* 2014; 121:272-9)

**A**NESTHESIA is a condition with patients exposed to numerous allergens that can induce anaphylaxis. Anaphylaxis is defined by the World Allergy Organization as the acute appearance of an illness with two or more of the following symptoms: involvement of the skin-mucosal tissue, reduced blood pressure, respiratory compromise, or gastrointestinal signs,<sup>1</sup> and it belongs to the larger classification of immediate hypersensitivity reactions (IHRs), which proceed through allergic or nonallergic mechanisms.<sup>2</sup> Life-threatening anaphylactic shock and inaugural cardiac arrest are the most severe forms of IHRs. Early recognition and immediate treatment are mandatory to ensure complete recovery of the patient. Despite appropriate treatment, their outcome may be poor, associated to complications up to death. Identification of the reaction mechanism is needed, as other causes such as drug toxicity, worsening of previous clinical

### What We Already Know about This Topic

- The diagnosis of life-threatening allergic reactions during anesthesia is usually made on the basis of hemodynamic instability and skin manifestations

### What This Article Tells Us That Is New

- A retrospective review of blood histamine and tryptase concentrations from 75 patients having life-threatening allergic reactions under anesthesia and 25 patients resuscitated from cardiac or other types of shock showed significantly higher concentrations of histamine and tryptase in the patients with allergic reactions and no effect of resuscitation procedures

condition, concomitant disease, or even medical error may be responsible for death.

Immediate allergic reactions proceed through allergen-specific immunoglobulin E binding, leading to acute

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degranulation of mast cell and basophil and release of stored mediators, particularly histamine and tryptase, which can be measured in plasma after reactions.<sup>3</sup> Histamine is released from mast cell and basophil and concentrations measured in plasma few minutes after reactions correlate with severity.<sup>4</sup> However, histamine can also be released from basophil *in vivo* through nonimmune, presumably toxic pathways, or *in vitro* by physical alteration of cell.<sup>5</sup> Tryptase originates from mast cell only. Two major forms are described such as  $\alpha$ -protryptase, which leaks continuously from mast cell and is present in normal plasma, and  $\beta$ -tryptase, which is stored in the granules and absent from normal plasma.<sup>6</sup> During acute reactions, increased  $\beta$ -tryptase concentrations correlate with the decrease in arterial blood pressure<sup>7</sup> and are associated with an allergic mechanism.<sup>8</sup> Total tryptase ( $\alpha$ -protryptase +  $\beta$ -tryptase) concentrations are remarkably stable with time in normal patients, with large interindividual variations.<sup>9</sup> Concentrations are transiently increased during systemic anaphylaxis,<sup>9</sup> and permanently increased in patients with systemic mastocytosis,<sup>10</sup> or with myeloid leukemia.<sup>11</sup> A combination of mediator measurements and allergic testing (skin tests and allergen-specific immunoglobulin E) is recommended to discriminate *a posteriori* allergic reactions from nonallergic ones and to identify the responsible agent.<sup>12\*</sup> Where skin testing is not available, or not performed because of poor outcome or death, mediator measurements in samples obtained rapidly after reactions may provide valuable information. However, to our knowledge, the effect of resuscitation manoeuvres on mediator concentrations is unknown and may impact the diagnostic value of histamine and tryptase measurements, particularly in case of very severe reactions or death.

The aim of the study was to evaluate the effect of heavy resuscitation on histamine and tryptase concentrations, to determine the thresholds associated with an allergic mechanism, and to estimate their diagnostic accuracy. For this purpose, we compared histamine and tryptase concentrations in patients with severe allergic IHR during anesthesia with those measured in patients resuscitated from shock due to other medical causes.

## Materials and Methods

The study was approved by the Institutional Review Board of the University Hospital of Reims, Reims, France (February 20, 2012).

### Study Population

**Group CONTROL.** Group CONTROL (cardiac arrest or septic/cardiogenic shock) was retrospectively constituted of 11 patients with profound hypotension due to septic

or cardiogenic shock and of 14 patients resuscitated from a cardiac arrest, without any relation with allergic event or anesthesia, in Nancy (France) University Hospital from April 2000 to December 2004. Diagnosis of septic shock<sup>13</sup> was supported by at least two of the following criteria: (1) a shock with a systolic arterial pressure less than 90 mmHg or decreased by 30% from baseline value, a heart rate greater than 90 beats/min,  $P_a\text{CO}_2$  less than 32 mmHg, and the need of vascular loading and use of dopamine or norepinephrine; (2) a urine output less than 30 ml/h; and (3) an infectious syndrome, with body temperature greater than 38°C or less than 36°C, spontaneous respiratory rate greater than 20 breaths/min, and leukocyte count greater than 12,000/mm<sup>3</sup> or less than 4,000/mm<sup>3</sup>. Diagnosis of cardiogenic shock was supported by altered ejection fraction of left ventricle with low cardiac output at echocardiography and invasive hemodynamic monitoring. Plasma samples obtained for other purposes during resuscitation were stored at -20°C and retrospectively used for mediator measurements.

**Group ALLERGY.** Patients with allergic IHRs during anesthesia were retrospectively included in Group ALLERGY (allergic immediate hypersensitivity reaction) from the *Groupe d'Etudes des Réactions Allergiques Peranesthésiques* multicenter French database recorded during the period from January 1, 2001 to December 31, 2002.<sup>14</sup> The inclusion criteria were the occurrence of a life-threatening reaction with cardiovascular collapse or cardiac arrest, the measurements of plasma histamine and tryptase within 2 h after the adverse reaction, and positive allergy tests to an administered drug or to latex, following the European recommendations.<sup>15</sup> Reactions were graded III (67 patients) or IV (8 patients) according to the Ring and Messmer severity scale (grade III = cardiovascular collapse, associated with tachycardia or bradycardia, arrhythmia, severe bronchospasm, with or without cutaneous signs and grade IV = circulatory arrest).<sup>16</sup> For all the patients, the medical history, especially the presence of atopy or asthma and the use of  $\beta$ -blocking agents, was recorded.

### Assays

Histamine concentrations were measured in duplicate in plasma obtained by gentle aspiration far from the blood cell layer, after centrifugation at 10° to 15°C, as previously described.<sup>5</sup> A radioimmunoassay (RIA Histamine; Beckman Coulter Immunotech, Marseille, France) was used, after alkylation of histamine according to the manufacturer's instructions. The normal values used in the laboratory are less than 6 nmol/l. In our hands, the minimal detectable concentration is less than 2 nmol/l, and the between-assay coefficient of variation is 7.4% for a mean concentration equal to 5.4 nmol/l and 9% for 13.8 nmol/l. Total tryptase concentrations were measured in serum or plasma by using an automated fluoroenzyme immunoassay (UniCAP Tryptase; ThermoFisher Scientific, Phadia S.A.S., Saint Quentin en Yvelines, France) with normal values less than 12.5  $\mu\text{g/l}$ . The minimal detectable concentration is 1  $\mu\text{g/l}$ , and the

\* National Institutes for Health and Clinical Excellence. Anaphylaxis. Assessment to confirm an anaphylactic episode and the decision to refer after emergency treatment for a suspected anaphylactic episode. NICE clinical guideline134. Available at: <http://www.nice.org.uk/nice-media/live/13626/57537/57537.pdf>. Accessed May 17, 2013.

between-assay coefficient of variation is 7% for a 7.4 µg/l concentration and 6.4% for a 25 µg/l.

### Statistical Analysis

The sample sizes were based on available data. All data were used for comparison analyses. Continuous variables were presented as mean ± SD or median (range) as appropriate. Categorical variables were presented as numbers and percentages. Statistical analysis was performed with Systat 11 (Systat Software Inc., San Jose, CA) and MedCalc 11.6.0 (MedCalc Software bvba, Ostend, Belgium). Continuous variables were compared with the Student *t* test or the Mann–Whitney *U* test. Repeated-measures analysis was performed with the non-parametric Friedman test. Categorical variables were compared with the Pearson chi-square test or Fisher exact test. Receiver operating characteristic (ROC) curves were generated with SigmaPlot 12.3 (Systat Software Inc.).<sup>17</sup> Areas under ROC curves were calculated and compared as recommended,<sup>18</sup> and the optimal threshold values were determined at the maximization of the Youden Index. Accuracy of results was provided by respective 95% CIs. Bayes' Theorem for conditional probabilities was used to calculate the positive and negative predictive values of tests. All hypotheses were two-tailed tested. A *P* value less than 0.05 indicated statistical significance.

### Results

One hundred patients were included in the study, 25 patients in Group CONTROL and 75 patients in Group ALLERGY. The demographic characteristics are summarized in table 1.

In Group CONTROL (8 women and 17 men), one patient had a history of atopy and four patients had asthma. Fourteen patients had cardiac arrest (grade IV), due to anoxia in five patients, ventricular fibrillation in three, myocardial infarction in two, cardiac insufficiency in two, cerebral edema in one, and pneumothorax in one, and 11 patients had cardiovascular collapse (grade III) due to septic shock. Amines were administered to 22 patients (adrenaline: 16; noradrenaline: 3; and dopamine: 4). All the patients with cardiac arrest had external cardiac massage, lasting more than

5 min in nine patients, and four patients had external electric shock. Fifteen patients died secondarily (nine patients with cardiac arrest and six patients with septic shock).

In Group ALLERGY (55 women and 20 men; table 1), nine patients had a history of atopy and three patients had asthma. Eight patients presented with cardiac arrest (grade IV reaction) and 67 with cardiovascular collapse (grade III). The agents administered before the reaction and giving positive skin tests (*n* = 68) or positive specific immunoglobulin E (*n* = 7, skin tests not done) were the following: neuromuscular-blocking agents in 47 patients (suxamethonium: 21; rocuronium: 12; atracurium: 6; vecuronium: 4; *cis*-atracurium: 2; pancuronium: 2); antibiotics in 13 (cephalosporine: 8; amoxicilline: 5); latex in 12; hydroxyl-ethyl starch, fluid gelatin, and patent blue V in 1 case each. Among the patients with cardiac arrest, four patients received external cardiac massage, two had external electric shock, and an intraaortic balloon with counterpulsation was inserted in one.

There was no significant difference between Group CONTROL and Group ALLERGY with regard to age, history of atopy, or asthma. Female sex was more represented in Group ALLERGY. History of β-blocking drug use was more frequent in Group CONTROL (table 1).

In Group CONTROL, four successive plasma samples were assayed for 21 patients, three samples for 3 patients, and one sample for 1 patient. The samples were classified as: T0: less than 1 h after the occurrence of cardiac arrest or shock (*n* = 25); T1: approximately 30 min after T0 (*n* = 24); T2: approximately 2 h after T0 (*n* = 24); and T3: the day after (at least 12 h) (*n* = 21). In two patients, histamine measurements were not done at one time point and in one patient at all the time points, due to insufficient plasma volume. The mean histamine and tryptase concentrations appeared within the normal range and remained unchanged throughout the resuscitation period (table 2). Two patients with cardiac arrest had increased concentrations of histamine (36.5 and 13.4 nmol/l, respectively). Another patient had increased tryptase concentrations in the three samples after cardiac arrest (15.2, 19.7, and 15.3 µg/l, respectively) as well as the day after (14 µg/l). Concentrations were significantly higher in patients with cardiac arrest than in those with shock for histamine (*P* = 0.008) but not for tryptase (table 3).

Histamine and tryptase concentrations were significantly higher in Group ALLERGY than in Group CONTROL (*P* < 0.026 and *P* < 0.003, respectively) (table 3 and fig. 1). A large dispersion of values was observed (see table, Supplemental Digital Content 1, <http://links.lww.com/ALN/B49>, which indicates the individual concentrations of histamine and tryptase, together with the characteristics of the patients and responsible agents). No significant differences related to the severity grade were observed for histamine (*P* = 0.45) or tryptase (*P* = 0.066) concentrations in Group ALLERGY (table 3).

**Table 1.** Comparison of the Characteristics of the Patients from Group ALLERGY (Allergic Immediate Hypersensitivity Reaction) and from Group CONTROL (Cardiac Arrest or Septic/Cardiogenic Shock)

|                   | Group ALLERGY (n = 75) | Group CONTROL (n = 25) | <i>P</i> Value |
|-------------------|------------------------|------------------------|----------------|
| Age (y)           | 49.8 ± 16.0            | 56.1 ± 20.5            | 0.118*         |
| Ratio female/male | 55/20                  | 8/17                   | 0.001†         |
| Atopy             | 9 (12%)                | 1 (4%)                 | 0.444‡         |
| Asthma            | 3 (4%)                 | 4 (16%)                | 0.063‡         |
| β-blocker use     | 3 (4%)                 | 5 (20%)                | 0.022‡         |

Values are mean ± SD or number of patients (female/male) or number (% of group).

Comparisons were performed with: \* Student *t* test; † Pearson chi-square test; and ‡ Fisher exact test.

Receiver operating characteristic curves<sup>17</sup> were generated from histamine or tryptase concentrations to differentiate Group ALLERGY from Group CONTROL (fig. 2). The areas under ROC curves approached 1 and were not significantly different between histamine and tryptase ( $P = 0.45$ ). For histamine concentrations, the optimal threshold was calculated as 6.35 nmol/l. Using this threshold, sensitivity was calculated from Group ALLERGY and specificity from Group CONTROL as 90.7 and 91.7%, respectively (table 4). For comparison, sensitivity was calculated as 84% and specificity as 91.7% with the 9 nmol/l threshold. For tryptase, the optimal threshold was 7.35  $\mu\text{g/l}$ , allowing 92% sensitivity and 92% specificity (table 4). Using the upper level of normal values (12.5  $\mu\text{g/l}$ ), sensitivity was calculated as 82.7% and specificity 96%, and respectively, 68 and 100% with a threshold at 25  $\mu\text{g/l}$  (table 4). Association of histamine and tryptase measurements moderately increased sensitivity (93.3%) and reduced specificity (83.3%).

**Table 2.** Group CONTROL: Plasma Concentrations of Histamine and Tryptase during Resuscitation of Patients with Cardiac Arrest or Septic/Cardiogenic Shock

|                              | T0                                | T1                                | T2                                | T3                                | P Value* |
|------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|----------|
| Histamine (nmol/l)           | n = 24<br>4.3 ± 7.4<br>(0.9–36.5) | n = 21<br>4.1 ± 7.5<br>(0.6–36.2) | n = 23<br>4.2 ± 9.9<br>(0.8–49.2) | n = 20<br>2.2 ± 1.6<br>(1.0–8.0)  | 0.34     |
| Tryptase ( $\mu\text{g/l}$ ) | n = 25<br>4.0 ± 3.1<br>(1.0–15.2) | n = 24<br>4.4 ± 3.8<br>(1.0–19.7) | n = 24<br>4.3 ± 3.2<br>(1.0–15.3) | n = 21<br>4.0 ± 2.8<br>(1.0–14.0) | 0.58     |

Values are mean ± SD (range).

\* Friedman test for nonparametric repeated measures.

T0 = <1 h after event; T1 = 30 min after T0; T2 = 2 h after T0; T3 = at least 12 h after event.

## Discussion

This study demonstrates that resuscitation manoeuvres do not modify histamine and tryptase concentrations in patients with cardiac arrest or profound hypotension unrelated to immediate hypersensitivity. Only patients with allergic IHR demonstrated significant increases in mediator concentrations. Optimal thresholds determined by ROC curves indicated that mediator concentrations exceeding the normal range were highly indicative of allergic events.

In the current study, Group CONTROL and Group ALLERGY were constituted from retrospective data. The number of patients in Group CONTROL was limited by the difficulty to obtain blood samples in unattended emergency conditions. Despite shorter inclusion period, Group ALLERGY was more numerous, due to inclusion from more centers and to recommendations for blood sampling by anesthesiologists and for allergy testing in France. The two groups presented with clinical events of similar severity and received amines and external cardiac massage or electric shock as appropriate. They were similar for age and history of atopy or asthma, but differed for sex distribution, as IHRs are more frequently observed in female,<sup>19,20</sup> and for pre-existing cardiovascular condition, as shown by the higher frequency of  $\beta$ -blocking drug use in Group CONTROL.

Mediator concentrations were significantly different between the two groups. In Group CONTROL, mean histamine and mean total tryptase concentrations did not vary throughout the resuscitation period and afterward. Mean histamine concentrations stood within the normal range (<6 nmol/l) although 8% of subjects had moderate increases, indicating a rare effect of the clinical condition or of resuscitation manoeuvres. The values measured in the other 92% were

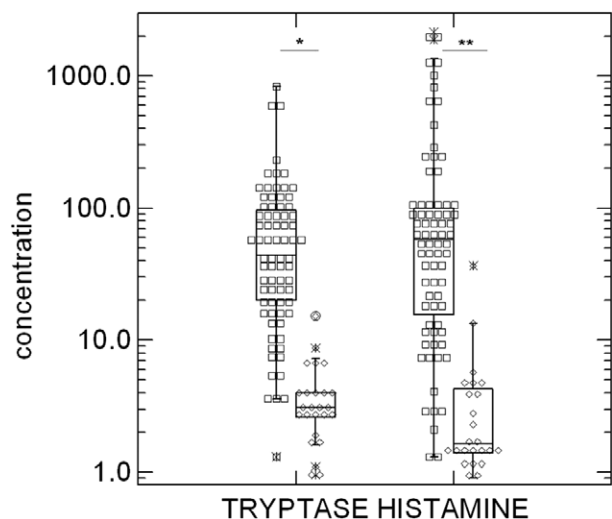
**Table 3.** Histamine and Tryptase Concentrations in 75 Patients with Allergic Immediate Hypersensitivity Reactions (Group ALLERGY) and in 25 Patients with Cardiac Arrest or Septic/Cardiogenic Shock Unrelated to Allergy or Anesthesia (Group CONTROL, Sample Obtained <1 h after the Event), According to the Severity of the Reaction

|                              | Group CONTROL | Group ALLERGY                | P Value*                          |
|------------------------------|---------------|------------------------------|-----------------------------------|
|                              | n             | n                            |                                   |
| Histamine (nmol/l)           |               |                              |                                   |
| All patients                 | 24†           | 75                           | 0.026                             |
|                              |               | 4.2 ± 7.3<br>1.6 [0.9–36.5]  | 190.3 ± 403.1<br>58.0 [1.3–2,140] |
| With shock                   | 11            | 67                           |                                   |
|                              |               | 1.7 ± 1.2<br>1.4 [0.9–5.2]   | 207 ± 423<br>59.8 [1.3–2,140]     |
| With cardiac arrest          | 13†           | 8                            |                                   |
|                              |               | 6.4 ± 9.6<br>3.7 [1–36.5]    | 48.1 ± 38.1<br>38.1 [3–100]       |
| Tryptase ( $\mu\text{g/l}$ ) |               |                              |                                   |
| All patients                 | 25            | 75                           | 0.003                             |
|                              |               | 3.9 ± 3.1<br>3.1 [0.95–15.2] | 86.5 ± 134.3<br>44.1 [1.3–835]    |
| With shock                   | 11            | 67                           |                                   |
|                              |               | 2.9 ± 0.9<br>2.8 [0.95–4.2]  | 78.6 ± 125<br>39.8 [1.3–835]      |
| With cardiac arrest          | 14            | 8                            |                                   |
|                              |               | 4.8 ± 3.9<br>3.5 [0.95–15.2] | 153 ± 196<br>93.3 [12.7–629]      |

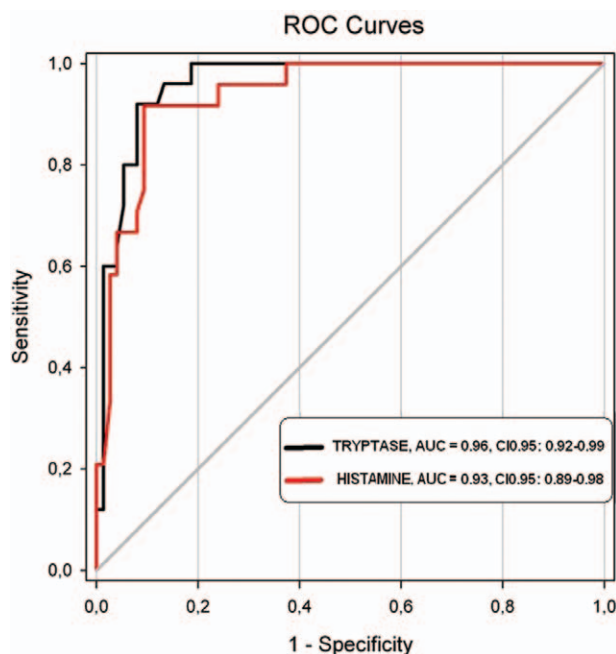
Values are mean ± SD, and median [range]; n is the number of cases.

\* Student *t* test; † histamine was not measured in one patient at this time point.





**Fig. 1.** Individual values and *box plots* of tryptase ( $\mu\text{g/l}$ ) and histamine ( $\text{nmol/l}$ ) concentrations in Group ALLERGY (allergic immediate hypersensitivity reaction) (*squares*) and in Group CONTROL (cardiac arrest or septic/cardiogenic shock) (*diamonds*) (logarithmic scale). Groups were significantly different. \* $P = 0.003$ ; \*\* $P = 0.026$ .



**Fig. 2.** Receiver operating characteristic (ROC) curves and corresponding areas under the curve (AUC) with 95% CI (0.95) for tryptase and histamine testing for allergy. Sensitivity = percentage of patients with mediator concentrations exceeding the threshold among patients with proved allergic shock (true positives); (1-specificity) = percentage of patients with mediator concentrations exceeding the threshold among patients with shock unrelated to allergy (false positives).

consistent with a previous study of 35 uneventfully anesthetized controls.<sup>3</sup> Similarly, total tryptase concentrations stood in the normal range ( $<12.5 \mu\text{g/l}$ ) for 96% of subjects, whereas one subject showed a permanent, moderate increase, possibly

due to latent mastocytosis, which could have worsened his condition.<sup>21</sup> The results were consistent with a report of 120 uneventfully anesthetized controls, indicating total tryptase concentrations of  $5.01 \pm 4.52 \mu\text{g/l}$  before and  $4.46 \pm 3.80 \mu\text{g/l}$  after anesthesia and surgery, and evidencing increased preoperative tryptase in 5.8% of patients.<sup>22</sup> Thus, it can be concluded that resuscitation had limited, if any, effect on histamine concentrations, and none for total tryptase.

In Group ALLERGY, histamine and tryptase concentrations were considerably increased compared with Group CONTROL. A large dispersion of histamine concentrations was observed, possibly due to its short half-life in plasma (15 to 20 min),<sup>5</sup> as the time delay elapsed between reaction and blood sampling varied between patients from few minutes to 2 h. No correlation with the severity of signs could be demonstrated, in contrary to challenge studies of minor or moderate reactions showing small histamine increases in which early sampling (1 to 2 min) did not impair comparison.<sup>4</sup> In the current study, it is plausible that sampling was done later in the most severe reactions (cardiac arrest) due to longer-lasting treatment. The correlation of tryptase concentrations with the severity grade did not reach significance, possibly due to the small number of cases with grade 4 reactions. Compared with histamine, tryptase half-life is long (90 to 120 min), but its peak is delayed<sup>6</sup> and may not be obtained in early samples, which could account for variability, as could possible interindividual differences in the amounts releasable by mast cell.<sup>23</sup>

Receiver operating characteristic curves were generated to determine the optimal mediator thresholds discriminating between allergic reactions and other medical events. Areas under ROC curve were approximately 1, indicating high diagnostic performances of tests. Equal areas indicating equal diagnostic efficacy were evidenced for histamine and tryptase.<sup>18</sup> The optimal threshold for histamine (6.35 nmol/l) corresponded to the upper normal value and allowed 90.7% sensitivity and 91.7% specificity. The optimal threshold for tryptase (7.35  $\mu\text{g/l}$ ) appeared lower than the upper normal level used in the laboratory (12.5  $\mu\text{g/l}$ ) and was associated with 92% sensitivity and 92% specificity.

When sensitivity and specificity of a test have been determined, respectively, from disease and nondisease cohorts, the positive and negative predictive values of the test can be obtained from conditional probabilities. Such calculations necessitate the assessment of the frequency of the disease in the population of interest. A large French multicenter epidemiological study indicated 1,094 allergic reactions and 76 nonallergic ones from 1,170 patients presenting with cardiovascular collapse or cardiac arrest during anesthesia,<sup>19</sup> which gives a pretest probability of allergy of 0.935 in that population. Using this value in the Bayes' Theorem for conditional probabilities, the positive predictive value indicating an allergic mechanism for the observed reaction was 99.4% for histamine or 99.4% for tryptase with our optimal thresholds, and the negative

**Table 4.** Diagnostic Performances of Plasma Histamine and Tryptase Measurements for the Diagnosis of Allergic Immediate Hypersensitivity, According to Different Thresholds

|                          | Threshold | Se %<br>(95% CI) | Sp %<br>(95% CI) |
|--------------------------|-----------|------------------|------------------|
| Histamine<br>(nmol/l)    | 6.35      | 90.7 (81.7–96.1) | 91.7 (73.0–98.9) |
|                          | 9         | 84.0 (73.7–91.4) | 91.7 (73.0–98.9) |
| Tryptase<br>( $\mu$ g/l) | 7.35      | 92.0 (83.4–97.0) | 92.0 (73.9–99.0) |
|                          | 12.5      | 82.7 (73.7–91.4) | 96.0 (79.6–99.9) |
|                          | 25        | 68.0 (56.2–78.3) | 100 (86.2–100)   |

Performances are expressed as percentage and 95% CI.

Se = sensitivity: percentage of patients with mediator concentrations exceeding the threshold among patients with proved allergic shock; Sp = specificity: percentage of patients with mediator concentrations not exceeding the threshold among patients with shock unrelated to allergy.

predictive values 40.7 or 44.3%, respectively. This means that a patient presenting with shock or cardiac arrest during anesthesia together with mediator concentrations exceeding the thresholds has a 99.4% probability to have suffered an allergic event. On the contrary, a patient with similar clinical presentation but without increased mediator concentrations cannot be diagnosed as nonallergic: due to the low negative predictive values of the tests, both diagnoses are possible.

Increasing the thresholds up to or over the upper normal values did not modify the positive predictive value of histamine (99.4% at 9 nmol/l), and marginally increased it for tryptase (99.7% at 12.5  $\mu$ g/l and 100% for 25  $\mu$ g/l), whereas the negative predictive values were considerably reduced (28.6, 27.9, and 17.8%, respectively).

To our knowledge, no scientifically validated threshold had been previously reported for histamine. Surveys of reactions of severity grades 1 through 4 during anesthesia using laboratory thresholds of 9 or 11 nmol/l calculated 78.2 or 71% sensitivity.<sup>20,24</sup> In the current study, at 9 nmol/l, sensitivity appeared higher (84%), which can be explained by the higher severity of the studied reactions. Thresholds reported for tryptase from ROC curves in other clinical settings, such as patients brought to the emergency room,<sup>25</sup> or severe reactions after challenge with hymenoptera venom<sup>26</sup> were 8.23 and 9  $\mu$ g/l and allowed 94.1 and 55% sensitivity, respectively.<sup>25,26</sup> Authors emphasized the interest of comparison with baseline tryptase concentrations,<sup>25–27</sup> which were not available in the current study. In two studies, combined measurements of histamine and tryptase substantially increased sensitivity for moderate reactions,<sup>26,28</sup> whereas only a marginal increase was obtained in the current study of severe reactions.

For reactions during anesthesia, the threshold for obviously increased total tryptase concentrations was arbitrarily set at 25  $\mu$ g/l,<sup>12</sup> to avoid false-positive values due to unapparent mastocytosis. Several studies using this cutoff calculated sensitivity ranging from 60 to 64%,<sup>20,24</sup> which is consistent with the 68% value obtained in the current study. Although interesting for its positive predictive value of

100%, confirming previous results,<sup>24</sup> this threshold appears too high to achieve sufficiently high sensitivity. Permanently increased tryptase due to mastocytosis can be ruled out by measurements in a baseline sample obtained before the reaction, or several hours afterwards.<sup>27</sup>

The current study demonstrates that the thresholds for histamine and tryptase measured during life-threatening reactions should be reduced, even when heavy resuscitation manoeuvres have been performed. With the optimal calculated thresholds, or using the upper level of the normal range, positive predictive values were calculated as at least 99.4%, indicating that virtually all acute hypotensive reactions or cardiac arrest associated with mediator concentrations exceeding the thresholds were allergic events. These results were obtained from living patients, and not from postmortem measurements. For plasma samples obtained postmortem, during anatomic verification, a much higher tryptase threshold (50  $\mu$ g/l in femoral blood) has been reported.<sup>29</sup> Cell lysis in tissue and blood occurs progressively after death, leading to increased tryptase concentrations that parallel the time elapsed between death and blood sampling, explaining the large range of concentrations reported.<sup>30</sup> Moreover, blood is heavily hemolysed, and basophil is disrupted, precluding reliable histamine measurements. Thus, it seems advisable not to rely on postmortem measurements, but to withdraw blood samples before stopping resuscitation, or within few minutes after death.

In the current study, we did not address the issue on nonallergic hypersensitivity reactions. These reactions are clinically undistinguishable from allergic ones, even though usually less severe,<sup>14</sup> they are defined by negative allergy testing and account for approximately 30% of reactions.<sup>19</sup> Nonallergic hypersensitivity reactions may be related to numerous mechanisms,<sup>31</sup> including nonspecific histamine release due to drug toxicity to basophil,<sup>32</sup> and large epidemiological studies of reactions occurring during anesthesia indicated increased histamine concentrations (>9 nmol/l) in 40% of these events.<sup>14</sup> More puzzling was the finding of increased tryptase concentrations (>25  $\mu$ g/l) in 4 to 7% of them.<sup>14,19</sup> However, for numerous drugs, in contrary to neuromuscular-blocking agents or latex, the diagnostic value of skin tests is poor, and specific immunoglobulin E assays are lacking. In our opinion, these few cases could be unidentified allergic reactions to agents for which allergy tests are poorly reliable.

Finally, in patients who die during anesthesia despite prolonged resuscitation, provided that blood samples be obtained before death, the thresholds calculated in the current study, 6.25 nmol/l for histamine or 7.35  $\mu$ g/l for total tryptase, allowed *a posteriori* diagnosis of allergic immediate hypersensitivity as the mechanism of the event with high reliability. These findings are especially important for patients who cannot receive allergic skin testing

and may have important forensic consequences for the anesthesiologist.

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## Competing Interests

Dr. Laroche declares payment of congress fees by ThermoFisher Scientific (Saint Quentin en Yvelines, France). The other authors declare no competing interests.

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## References

1. Simons FER, Arduzzo LRF, Bilo MB, El-Gamal YM, Ledford DK, Ring J, Sanchez-Borges M, Senna GE, Sheikh A, Thong BY: World Allergy Organization guidelines for the assessment and management of anaphylaxis. *World Allergy Organ J* 2011; 4:13–37
2. Johansson SGO, O'B Hourihane J, Bousquet J, Brujnzeel-Koomen C, Dreborg S, Haahntela T, Kowalski ML, Mygind N, Ring J, van Cauwenberge P, van Hage-Hamsten M, Wüthrich B: A revised nomenclature for allergy. An EAACI position statement from the EAACI nomenclature task force. *Allergy* 2001; 56:813–24.
3. Laroche D, Vergnaud MC, Sillard B, Soufarapis H, Bricard H: Biochemical markers of anaphylactoid reactions to drugs. Comparison of plasma histamine and tryptase. *ANESTHESIOLOGY* 1991; 75:945–9
4. Halmerbauer G, Hauk P, Forster J, Urbanek R, Kaufmehl K, Koller DY: *In vivo* histamine release during the first minutes after deliberate sting challenges correlates with the severity of allergic symptoms. *Pediatr Allergy Immunol* 1999; 10:53–7
5. Laroche D, Dubois F, Gérard JL, Lefrançois C, André B, Vergnaud MC, Dubus L, Bricard H: Radioimmunoassay for plasma histamine: A study of false positive and false negative values. *Br J Anaesth* 1995; 74:430–7
6. Schwartz LB: Diagnostic value of tryptase in anaphylaxis and mastocytosis. *Immunol Allergy Clin North Am* 2006; 26:451–63
7. Watkins J: Tryptase release and clinical severity of anaesthetic reactions. *Agents Actions* 1992; 36:C203–205
8. Fisher MM, Baldo BA: Mast cell tryptase in anaesthetic anaphylactoid reactions. *Br J Anaesth* 1998; 80:26–9
9. Schwartz LB, Bradford TR, Rouse C, Irani AM, Rasp G, Van der Zwan JK, Van der Linden PW: Development of a new, more sensitive immunoassay for human tryptase: Use in systemic anaphylaxis. *J Clin Immunol* 1994; 14:190–204
10. Schwartz LB, Sakai K, Bradford TR, Ren S, Zweiman B, Worobec AS, Metcalfe DD: The alpha form of human tryptase is the predominant type present in blood at baseline in normal subjects and is elevated in those with systemic mastocytosis. *J Clin Invest* 1995; 96:2702–10
11. Sperr WR, Jordan JH, Baghestanian M, Kiener HP, Samorapoompichit P, Semper H, Hauswirth A, Scherthaner GH, Chott A, Natter S, Kraft D, Valenta R, Schwartz LB, Geissler K, Lechner K, Valent P: Expression of mast cell tryptase by myeloblasts in a group of patients with acute myeloid leukemia. *Blood* 2001; 98:2200–9
12. Laxenaire MC: Reducing the risk of anaphylaxis during anaesthesia. Abbreviated text. *Rev Fr Allergol Immunol Clin* 2002; 42:650–65
13. Lever A, Mackenzie I: Sepsis: Definition, epidemiology, and diagnosis. *BMJ* 2007; 335:879–83
14. Mertes PM, Laxenaire MC: Les membres du GERAP: Anaphylactic and anaphylactoid reactions occurring during anaesthesia in France. Seventh epidemiologic survey (January 2001–December 2002). *Ann Fr Anesth Réanim* 2004; 23:1133–43 (French)
15. Mertes PM, Laxenaire MC, Lienhart A, Aberer W, Ring J, Pichler WJ, Demoly P; Working Group for the SFAR; ENDA; the EAACI Interest Group on Drug Hypersensitivity: Reducing the risk of anaphylaxis during anesthesia. Guidelines for clinical practice. *J Invest Allergol Clin Immunol* 2005; 15:91–101
16. Ring J, Messmer K: Incidence and severity of anaphylactoid reactions to colloid volume substitutes. *Lancet* 1977; 1:466–9
17. Hanley JA, McNeil BJ: The meaning and use of the area under a receiver operating characteristic (ROC) curve. *Radiology* 1982; 143:29–36
18. DeLong ER, DeLong DM, Clarke-Pearson DL: Comparing the areas under two or more correlated receiver operating characteristic curves: A nonparametric approach. *Biometrics* 1988; 44:837–45
19. Mertes PM, Alla F, Tréchet P, Auroy Y, Jouglu E; Groupe d'Etudes des Réactions Anaphylactoïdes Peranesthésiques: Anaphylaxis during anesthesia in France: An 8-year national survey. *J Allergy Clin Immunol* 2011; 128:366–73
20. Dong SW, Mertes PM, Petitpain N, Hasdenteufel F, Malinovsky JM; Members of the GERAP: Hypersensitivity reactions during anesthesia. Results from the ninth French survey (2005–2007). *Minerva Anestesiologica* 2012; 78:868–78
21. Ludolph-Hauser D, Ruëff F, Fries C, Schöpf P, Przybilla B: Constitutively raised serum concentrations of mast-cell tryptase and severe anaphylactic reactions to Hymenoptera stings. *Lancet* 2001; 357:361–2
22. Garvey LH, Bech B, Mosbech H, Krøigaard M, Belhage B, Husum B, Poulsen LK: Effect of general anesthesia and orthopedic surgery on serum tryptase. *ANESTHESIOLOGY* 2010; 112:1184–9
23. Genovese A, Stellato C, Marsella CV, Adt M, Marone G: Role of mast cells, basophils and their mediators in adverse reactions to general anesthetics and radiocontrast media. *Int Arch Allergy Immunol* 1996; 110:13–22
24. Malinovsky JM, Decagny S, Wessel F, Guilloux L, Mertes PM: Systematic follow-up increases incidence of anaphylaxis during adverse reactions in anesthetized patients. *Acta Anaesthesiol Scand* 2008; 52:175–81

25. Enrique E, García-Ortega P, Sotorra O, Gaig P, Richart C: Usefulness of UniCAP-Tryptase fluoroimmunoassay in the diagnosis of anaphylaxis. *Allergy* 1999; 54:602–6
26. Brown SG, Blackman KE, Heddle RJ: Can serum mast cell tryptase help diagnose anaphylaxis? *Emerg Med Australas* 2004; 16:120–4
27. Borer-Reinhold M, Haeberli G, Bitzenhofer M, Jandus P, Hausmann O, Fricker M, Helbling A, Müller U: An increase in serum tryptase even below 11.4 ng/mL may indicate a mast cell-mediated hypersensitivity reaction: A prospective study in Hymenoptera venom allergic patients. *Clin Exp Allergy* 2011; 41:1777–83
28. Lin RY, Schwartz LB, Curry A, Pesola GR, Knight RJ, Lee HS, Bakalchuk L, Tenenbaum C, Westfal RE: Histamine and tryptase levels in patients with acute allergic reactions: An emergency department-based study. *J Allergy Clin Immunol* 2000; 106(1 Pt 1):65–71
29. Edston E, Eriksson O, van Hage M: Mast cell tryptase in post-mortem serum-reference values and confounders. *Int J Legal Med* 2007; 121:275–80
30. Way MG, Baxendine CL: The significance of post mortem tryptase levels in supporting a diagnosis of anaphylaxis. *Anaesthesia* 2002; 57:310–1
31. Farnam K, Chang C, Teuber S, Gershwin ME: Nonallergic drug hypersensitivity reactions. *Int Arch Allergy Immunol* 2012; 159:327–45
32. Fisher M, Baldo BA: Anaphylaxis during anesthesia: Current aspects of diagnosis and prevention. *Eur J Anaesth* 1994; 11:263–84