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Mast cell activation during suspected perioperative hypersensitivity: a need for paired samples analysis

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Abstract

Background:

Perioperative hypersensitivity (POH) reactions constitute a significant clinical and diagnostic challenge. A transient increase in serum tryptase during POH reflects mast cell activation (MCA) and helps to recognize an underlying hypersensitivity mechanism.

Objective:

This study aims to determine the diagnostic performance of different tryptase decision thresholds based upon single and paired measurements to document MCA in suspected POH.

Methods:

Acute serum tryptase (aST) and baseline serum tryptase (bST) samples were obtained from patients referred to our outpatients' clinic because of clinical POH. Tryptase samples from controls were obtained before induction (Tt_0) and 1.5 hours after induction (Tt_1) in uneventful anesthesia. Different cut-off points for delta tryptase (ΔT) and the percentage increase of tryptase (%T) were calculated and compared to existing thresholds: aST > [1.2 x (bST) + 2] (consensus formula), aST >11.4 ng/mL and aST >14 ng/mL.

Results:

POH patients had higher bST and aST leves compared to controls (respectively 5.15 vs 2.28 ng/mL for bST and 20.30 vs 1.92 ng/mL for aST). The consensus formula and a ΔT of $\geq$3.2 ng/mL held the highest accuracies to document MCA in POH (resp. 81 and 82%). A bST of > 8 ng/mL was present in 4% of controls, 5% of grade 1 POH, 24% of grade 2 POH, 15% of grade 3 POH and 17% of grade 4 POH.

Conclusion:

Our data endorse the consensus formula for detection of MCA in POH. Furthermore, it shows that a bST of > 8ng/mL was associated with occurrence of anaphylaxis.

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Clinical trial registration number: B300201837509

Highlight box

What is already known about this topic?

Measuring acute tryptase during an adverse perioperative event can help to recognize a mast cell related hypersensitivity.

What does this article add to our knowledge?

Paired samples are useful for recognizing a hypersensitivity reaction and detecting an underlying mast cell related predisposing condition.

How does this study impact current management guidelines?

Our data endorse the reliability of the [1.2 x (bST) + 2] consensus formula.

Key words

Perioperative hypersensitivity, tryptase, anesthesia, anaphylaxis, acute tryptase, baseline tryptase, mast cell, mast cell activation

Abbreviations

Abbreviations	
%ΔT	Percentage of tryptase increase over baseline tryptase
ΔT	Tryptase increase over baseline tryptase
AAAAI	American Academy of Allergy, Asthma, and Immunology
aST	Acute serum tryptase
AUC	Area under the curve
bST	Baseline serum tryptase
EAACI	European Academy of Allergy and Clinical Immunology
ELISA	Enzyme-Linked Immuno Sorbent Assay
HαT	Hereditary alpha-tryptasemia
ISM	Indolent systemic mastocytosis
MC	Mast cell
MCA	Mast cell activation
NPV	Negative predictive value
POH	Perioperative hypersensitivity
PPV	Positive predictive value
ROC	Receiver operating characteristic
SM	Systemic mastocytosis
WHO	World Health Organization

Introduction

Perioperative hypersensitivity (POH) reactions constitute a significant clinical and diagnostic challenge, a consequence of heterogeneous clinical presentations, and distinct underlying pathophysiological mechanisms of mast cell (MC) and basophil degranulation[1]. Besides, several of the clinically indistinguishable manifestations can occur independently from MC and basophil degranulation but may result from anesthetic or surgical technique or the pharmacological properties of the multiple potential causes[1, 2]. Through the years, several clinical grading scores for hypersensitivity reactions and anaphylaxis have been proposed as a way of stratifying severity[2-5], some especially for suspected POH[1, 6-8]. Supplementary serological biomarkers can help in detecting MC or basophil implication.

Tryptase is almost exclusively produced in MCs, and is the most abundant prestored serine protease, with minimal contribution from basophils[9, 10]. In humans, four isoforms of tryptase have been described but only the α- and β-isoforms are secreted. A portion of the alpha- and β-monomeric pro-forms are released spontaneously at baseline from resting MCs, while the remaining pro-forms are converted to mature tryptase under alpha/beta-homotetramers or alpha/beta-heterotetramers. These tetramers are stored in specialized secretory granules and released during degranulation. Early immunoassays only detected mature tryptase, with positive results indicating recent MC activation but with insufficient sensitivity[11-13]. In the sandwich ELISA method described in 1994, the measured serum tryptase is the sum of pro- and mature forms of both the α- and β-isoforms and was henceforth called total tryptase[14-17]. Although a mature tryptase assay exists, in clinical practice we rely on the ELISA methods that measure the total tryptase. In such assays, in order to depict elevation of β-tryptase, one needs to analyze paired acute-baseline samples[11, 16, 17]. The foundations of the utility of serum tryptase as a biomarker of MC degranulation during anaphylaxis dates back to 1989[11]. Using an experimentally induced systemic anaphylaxis model, the authors showed that, in most patients, the peak level of tryptase occurs 1-2 hours after the precipitating event. The first tentative judgment criterion was an acute total tryptase equal to at least the double of the baseline value[16, 18]. Two decades later, an acute serum tryptase (aST) value exceeding [1.2 x baseline serum tryptase (bST) + 2] has been proposed as a consensus threshold to document clinically significant MC activation (MCA) and this has been adopted for use in the perioperative setting[1, 19, 20]. Despite the shift from mature to total tryptase assays, some authors relied on fixed single predetermined aST levels such as an aST exceeding 11.4 ng/mL (the

methods' upper reference limit defined as the 95 upper percentile of apparently healthy subjects)[20-22] or its earlier value of 14 ng/mL[8, 16, 23-25]. Laroche et al, defined a diagnostic threshold of 7.35 ng/mL of tryptase and 6.35 nmol/L of histamine to differentiate between patients with septic or cardiogenic shock and patients with life-threatening POH reactions. If the sample is obtained before death, this threshold could be a useful insight into the etiology of the reaction in forensic situations when a bST is not available[26].

Others have studied and used paired aST and bST levels as a proof of MCA, either by calculating the delta tryptase (ΔT = aST - bST) or the percentage increase over the bST ($\%\Delta T$ = (aST - bST)/bST). Cut-off points that have been suggested by past studies are a $\Delta T \geq 3$ ng/mL[21, 23] or a $\%\Delta T$ of $\geq 35\%$[20, 21].

As recently reviewed by Passia[27], at present the 2012 consensus formula has been evaluated on four occasions, including three studies on POH patients[20, 28, 29] and in one study in children who experienced anaphylaxis from various causes[30]. From the POH studies, it emerges that the sensitivity (Se), specificity (Sp), positive predictive value (PPV) and negative predictive value (NPV) of this consensus formula varies between 75-78%, 86-91%, 94-98% and 44-53%, respectively. Furthermore, it has been demonstrated that this consensus formula can depict MCA in patients with an aST below 11.4 ng/mL[20, 29].

The best way to define MCA in POH is still disputed. We hypothesize that a large study including paired tryptase samples from patients who experienced POH together with control samples obtained before and after uneventful anesthesia could help to resolve this dispute. Therefore, this study aims to determine the diagnostic performance of different tryptase decision thresholds based upon single and paired measurements to document MCA in suspected POH.

Methods

Study population

Data were collected retrospectively from 296 patients who were referred to our outpatients' clinic because of a suspected POH reaction and who had a paired aST and bST measurement between 01/2006 and 06/2020. Data were collected on demographics, clinical features, reaction severity and probable causes/agents. The diagnosis of a POH reaction was made according to the AAAAI/EAACI guidelines for anaphylaxis[31, 32]. Briefly, anaphylaxis was defined as an acute, life-threatening, systemic hypersensitivity reaction characterized by a rapid onset of skin and mucosal changes and/or airway, or circulatory problems. Severity of POH reactions was graded according the NAP6 score[8], as used in our previous publication[33]. All patients were subjected to allergic work-up which included specific IgE (sIgE), total IgE, skin prick tests, intradermal tests, basophil activation tests, and, when indicated graded drug provocation tests. In addition, we included paired tryptase measurements from 75 control patients who had an uneventful general anesthesia at the Antwerp University Hospital. These patients underwent surgery for various indications. Approval for this study was obtained from the local ethical committee (reference number B300201316408). Patients and controls signed an informed consent in accordance with the Declaration of Helsinki.

Tryptase

Sampling time-points of aST from patients with a POH ranged between 60 and 120 minutes after onset of symptoms. Baseline tryptase was acquired at least 24 hours after resolution of the reaction or later during allergy work up. Paired tryptase measurements from control patients were obtained at induction (Tt_0) and after approximately 1.5 hours of general anesthesia (Tt_1). Tryptase was measured by the FEIA ImmunoCAP (Phadia Thermo Fisher Scientific, Uppsala, Sweden) with an upper reference value of 11.4 ng/mL. Samples were further diluted if the titer exceeded more than 200 ng/mL.

We looked at proportion of individuals with a bST higher than 8, 11.4 and 20 ng/mL among control samples and POH patients. bST level >8 ng/mL is indicative for hereditary alpha-tryptasemia[34-36] and a bST > 20 ng/mL is a minor criterium for the diagnosis of systemic mastocytosis[37].

Performance of different decision thresholds for serum tryptase

To compare different decision thresholds for serum tryptase we applied a multi-step approach. First, we generated receiver operating characteristic (ROC) curves to determine the ideal cut-off point that discriminates between patients who experienced a POH and controls. These ROC curves were constructed for aST and Tt_1, ΔT (aST-bST and Tt_1-Tt_0) and percentage increase of tryptase [(aST-bST)/bST and (Tt_1- Tt_0)/ Tt_0].

After determination of the ideal cut-off point, we calculated the Se, Sp, PPV and NPV for aST, ΔT and percentage increase of tryptase. Furthermore, we also calculated Se, Sp, PPV and NPV for fixed cut-off points of 11.4 ng/mL[20, 21] and 14 ng/mL[8, 23, 24] and for the consensus formula [1.2 x (bST) + 2][19]. We also explored the performance of the different decision thresholds among the four severity grades.

Statistical analysis

Tryptase levels were expressed as median (range). Non-parametric Mann–Whitney U test was used to compare unpaired continuous variables. When more than 2 groups had to be compared Kruskal-Wallis test was used. Wilcoxon matched-pairs signed rank test was used to compare paired continuous variables. Categorical variables were expressed as frequencies and differences were evaluated using Chi-square test and a Fisher exact test when appropriate (frequency of variable <5)[38].

The accuracy of each threshold was calculated as followed:

Accuracy = true positive + true negative/ (true positive + false positive + true negative + fase negative)

Statistically significant differences between performances of different decision thresholds were obtained by comparing the ROC areas under the curve (AUC, see Figure 3). P values ≤ 0.05 were considered significant. Results were analyzed using PRISM 8 (GraphPad Software, San Diego, USA) and JMP Pro 14 (SAS software, Cary, USA).

Results

Allergy work-up

Of the 296 patients, 19 (6%) experienced a grade 1 reaction, 29 (10%) a grade 2 reaction, 171 (58%) a grade 3 reaction and 77 (26%) a grade 4 reaction. After diagnostic work-up a cause was identified in 225 of the 296 (76%) patients. The most frequent identified causes were NMBAs (39%, predominantly rocuronium 86%), antibiotics (14%, predominantly cefazolin 86%), chlorhexidine (8%), latex (6%) and others (8%, sugammadex, radiographic contrast media, propofol, paracetamol, ondansetron). A causative agent was not found in 71 or the 296 (24%) patients who had a clinical POH reaction. All patients had skin test, sIgE quantification, basophil activation test and challenges for the products they were exposed to and for which it was appropriate/available. Besides all patients were systematically tested for latex and chlorhexidine. Of these 71 patients, 4 (6%) experienced a grade 1 reaction, 15 (21%) a grade 2 reaction, 40 (56%) a grade 3 reaction and 12 (17%) a grade 4 reaction. In thirty-eight (54%) of the patients without a cause, a significant rise in serum tryptase was measured during the acute event that fulfilled the consensus formula [1.2 x (bST) + 2][19]. There was no difference in median age between patients and controls. There were 39% men in the patient group and 60% men in the controls ($p < .001$). Details on the demographics and the causes are summarized in table 1 and E1 of the online repository.

Performance of different methods to confirm mast cell activation (MCA)

The median bST and aST in the POH group was 5.15 ng/mL (1.00-66.00) and 20.30 ng/mL (2.20-429.00), respectively ($p < .001$). Median bST and aST, respectively, were higher in men (5.40 and 27.85 ng/mL) than in women (4.95 and 17.80 ng/mL) ($p = 0.02$ and 0.03). Measurements in the control group were respectively 2.28 ng/mL (0.04-19.38) and 1.92 ng/mL (0.44-14.00) for Tt_0 and Tt_1 and did not differ significantly (Figure 1). Note that the bST value in patients was significantly higher than Tt_0 and Tt_1 values in control individuals ($p < .001$). No significant gender-based differences in tryptase levels were seen in the control group for Tt_0 and Tt_1 for men (2.36 and 1.95 ng/mL) and for women (2.09 and 1.90 ng/mL).

Using the paired tryptase samples from patients and controls, ROC curves were generated for aST, ΔT and the percentage increase of tryptase to determine the ideal cut-off point to distinguish between patients and controls (Figure 2). The calculated ideal cut-off points were an aST of ≥ 5.1, a ΔT of ≥ 3.2 ng/mL and an increase of tryptase $\geq 85\%$. Because of the differences seen in aST between men and women, separate gender-dependent analyses for aST were performed. ROC curves generated cut-off values for aST of 5.1 in men and 6 in women.

However, these values fall within the interquartile range of the median values of bST for men and women that are respectively 4.10-7.23 ng/mL and 3.10-6.50 ng/mL.

Subsequently, we compared the diagnostic performance of a ΔT of ≥3.2 ng/mL, percentage tryptase increase of ≥85%, the consensus formula[19], aST >11.4 ng/mL and aST >14 ng/mL. Table 2 summarizes the Se, Sp, PPV and NPV for these different decision thresholds. Based on the AUC analyses (Figure 3) the threshold of ΔT of ≥3.2 ng/mL and the consensus formula [1.2 x (bST) + 2][19] had the best performances of respectively, 82% and 81%. There was no significant difference between the these two performences (Figure 3).

Performance for different severity grades

Table 4 summarizes the median bST, aST and ΔT values per severity grade. Baseline serum tryptase did not differ significantly among the different grades of severity. Acute serum tryptase and ΔT were incrementally higher in grade 1, grade 2, grade 3 and grade 4. Levels of aST and ΔT were significantly different between all grades except for grade 1 and 2 (Figure 4). A rise of sensitivity was seen for all thresholds, as grades of severity increased. All the decision thresholds were able to distinguish between control values and severity grades except for the aST >14 ng/mL cut-off. For this threshold, there was no difference between the number of controls meeting the threshold and rate of POH patients with a grade 1 reaction (Table 3).

Baseline serum tryptase

Four percent of controls had a bST of ≥8 ng/mL compared to 16% of POH patients (p = 0.007). In severity grades 2-4, significantly more patients demonstrated a bST of ≥ 8 ng/mL, respectively 24, 15 and 17% (p = 0.003; 0.009; 0.007) as compared to controls. This was not the case for POH patients with a grade 1 reaction (5%).

There were no significant differences between controls and patients in number of individuals with a bST between 11.4-20 ng/mL and a bST >20 ng/mL. Fifteen (5%) patients and 3 (4%) controls had a bST between 11.4-20 ng/mL and 3 (1%) patients and 0 (0%) controls had a bST above 20 ng/mL.

Further work-up (including bone marrow biopsy) for detection of a systemic mastocytosis (SM) was proposed to the three patients with a bST above 20 ng/mL. After work-up, one patient was diagnosed with indolent systemic mastocytosis (ISM) and another one met 2 minor WHO criteria for systemic mastocytosis (= pre-diagnostic form of SM)[37]. The third patient was lost to follow-up. One other patient displayed a pre-diagnostic form of SM. This patient had

295 evidence of spindle shaped mast cells in the bone marrow biopsy without fulfilling other WHO
296 major or minor criteria[37]. This patient had a bST level of 5 ng/mL.
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Discussion

Although the clinical utility of serum tryptase as a biomarker of MC degranulation during anaphylaxis was established more than three decades ago[11], there is room for improvement of decision threshold for MCA in POH[8, 19-21, 23, 24, 28, 39, 40] . Here, we explored the diagnostic performance of different tryptase cut-off values in a large group of 296 patients who were referred by the attending anesthetist for investigation of a possible POH and 75 control individuals who had uneventful general anesthesia for various indications.

From our analyses different conclusions emerge. First, our data show that in uncomplicated anesthesia there is a slight decrease in serum tryptase. This decrease has been observed previously in a study performed by Garvey et al. in orthopedic surgery patients and could be explained by the dilutional effect of fluid administration during anesthesia. Admittedly, in our study we cannot exclude such a dilutional effect. However, experimental studies indicate that such a dilutional effect leads to an underestimation of aST is unlikely[41]. Secondly, our data emphasize that the use of a single aST value does not accurately represent MCA during POH and it confirms that optimal determination of MCA during POH needs a paired sampling with measurement of both aST and bST. This is reflected by the higher accuracy of the paired sampling thresholds (ΔT >3.2 ng/mL; Consensus formula [1.2 x (bST) + 2]; > 85% increase) compared to single measurements (aST > 11.4 or 14 ng/mL). As already emphasized by others[20, 21, 41], an apparently normal aST, that is, a value below the manufacturers' upper reference value of 11.4 ng/mL, does not exclude MCA. This reference limit of tryptase is defined as the 95-upper percentile of a control population without signs of MCA. This limit has changed over time and depends on the method (e.g. 13.5 ng/mL to 11.4 ng/mL, and more recently to 11 ng/mL[16, 25, 42]), the platform performance variation[43] and the population characteristics[16, 44, 45]. Also, the ROC generated cut-off points for aST in men and women in our study fell within the interquartile range of the bST levels. This means that these aST cut-off points are not applicable for MCA in POH as normal bST levels could be mistaken for MCA. Although, the ΔT >3.2 ng/mL threshold had a higher accuracy than the consensus formula [1.2 x (bST) + 2], this difference was not statistically significant. These data endorse the consensus formula [1.2 x (bST) + 2] to depict MCA during POH.

In a second set of analyses, we focused on the diagnostic performance of the different thresholds in relation to the different severity grading scores[1, 8]. We found that higher grades of severity coincide with higher sensitivity for all the studied decision thresholds. This can be explained by incrementally higher values of aST in the higher grades of severity. Actually, paralleling the observation by others, we show an association between the magnitude of the

rise of aST and POH severity grade[20, 28, 33]. On the other hand, in absence of hypotension (grade 1 and 2 POH) , where the peak aST is less pronounced, paired tryptase sampling can contribute to identify MCA as these reactions are more prone to be overlooked as POH reactions. The higher sensitivity of the paired sampling methods will be more capable to detect smaller but significant tryptase changes that are indicative of MCA. This finding reinforces the message made by Malinovsky and colleagues to promote tryptase measurements in mild and unexplained reactions that might be overlooked as POH reactions during anesthesia[46].

Systematic measurement of bST also enables the identification of underlying conditions such as a clonal mast cell disorder and hereditary alpha-tryptasemia[34-36]. In our population, one patient was diagnosed with ISM and two had pre-diagnostic forms of SM (2 minor WHO criteria[37] and population of spindle shaped mast cells). Admittedly, a bST tryptase level of >20 ng/mL is not the only indication for pursuing the diagnosis of SM and it is increasingly recognized that normal tryptase levels do not rule out SM [37, 47, 48]. Therefore, it cannot be excluded that some patients with an underlying SM might have been missed[37, 49, 50]. Furthermore, in our study, patients with a POH had a significantly higher bST level compared to healthy controls. A bST > 8 ng/mL, which might be indicative of HαT, was more prevalent in severity grades 2-4 (respectively 24, 15 and 17%) compared to controls and grade 1 POH (respectively 4 and 5%). Previous data suggests that HαT patients are at higher risk for more severe spontaneous or Hymenoptera sting-triggered anaphylaxis[51]. Our data from POH samples show that a bST >8 ng/mL is more frequently associated with occurrence of anaphylaxis. Therefore, it will be interesting to study the role of HαT as risk-factor and its′ contribution to the severity of anaphylaxis in perioperative setting. Paired sampling avoids further pitfalls in patients with elevated bST due to various conditions such as myeloid hematologic disorders[52], cardiovascular or renal conditions[53-55].

There are some limitations of this study. First, one could argue that the absence of precise data about the timing of the sampling of the aST constitutes a limitation of our study. However, for many years, we have systematically promoted an aST sampling between 60 and 90 minutes after onset of the reaction and our findings run parallel with the observations in the adequate timing group described by Vitte et al[20]. In their study of 85 patients that had aST sampling between 30 and 120 min, the consensus formula attained a similar Se, Sp, PPV and NPV of 75%, 86%, 94% and 53%, respectively. Second, we did not measure the number of TPSAB1 gene copies for identification of HαT. Third, there was a statistically significant difference in the gender ratio between patients and controls. Therefore, the higher bST value in the

predominantly female patient population compared to mostly male control population could reflect a gender effect. However, studies investigating the influence of gender on bST have made different observations[56, 57]. These contrasting observations might have been influenced by different study populations. The reported higher bST values in men or women range around a magnitude 0.2 ng/mL, which is far less than the difference of 2.87 ng/mL seen between our patients and control individuals. Therefore, we are confident that our calculations and proposals are valid.

In conclusion, our data support the recent recommendation for paired acute and baseline sampling in POH and endorse the consensus formula to depict MCA in POH[20, 28, 29]. Furthermore, it shows that magnitude of the rise of aST is associated with higher severity grades in POH. Finally, we found higher levels of bST in patients with POH compared to controls and a bST of > 8ng/mL, which might be indicative of HαT, was associated with occurrence of anaphylaxis. Further studies are required to establish role of HαT as risk-factor for occurrence and/or severity of POH.

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References:

1. Garvey LH, Ebo DG, Mertes PM, Dewachter P, Garcez T, Kopac P, et al. An EAACI position paper on the investigation of perioperative immediate hypersensitivity reactions. Allergy 2019; 74:1872-84.
2. Ebo DG, Clarke RC, Mertes PM, Platt PR, Sabato V, Sadleir PHM. Molecular mechanisms and pathophysiology of perioperative hypersensitivity and anaphylaxis: a narrative review. Br J Anaesth 2019; 123:e38-e49.
3. Niggemann B, Beyer K. Time for a new grading system for allergic reactions? Allergy 2016; 71:135-6.
4. Brown SG. Clinical features and severity grading of anaphylaxis. J Allergy Clin Immunol 2004; 114:371-6.
5. Ring J, Messmer K. Incidence and severity of anaphylactoid reactions to colloid volume substitutes. Lancet 1977; 1:466-9.
6. Kroigaard M, Garvey LH, Gillberg L, Johansson SG, Mosbech H, Florvaag E, et al. Scandinavian Clinical Practice Guidelines on the diagnosis, management and follow-up of anaphylaxis during anaesthesia. Acta Anaesthesiol Scand 2007; 51:655-70.
7. Rose MA, Green SL, Crilly HM, Kolawole H. Perioperative anaphylaxis grading system: 'making the grade'. Br J Anaesth 2016; 117:551-3.
8. Cook TM, Harper NJN, Farmer L, Garcez T, Floss K, Marinho S, et al. Anaesthesia, surgery, and life-threatening allergic reactions: protocol and methods of the 6th National Audit Project (NAP6) of the Royal College of Anaesthetists. Br J Anaesth 2018; 121:124-33.
9. Castells MC, Irani AM, Schwartz LB. Evaluation of human peripheral blood leukocytes for mast cell tryptase. J Immunol 1987; 138:2184-9.
10. Jogie-Brahim S, Min HK, Fukuoka Y, Xia HZ, Schwartz LB. Expression of alpha-tryptase and beta-tryptase by human basophils. J Allergy Clin Immunol 2004; 113:1086-92.
11. Schwartz LB, Yunginger JW, Miller J, Bokhari R, Dull D. Time course of appearance and disappearance of human mast cell tryptase in the circulation after anaphylaxis. J Clin Invest 1989; 83:1551-5.
12. Wenzel S, Irani AM, Sanders JM, Bradford TR, Schwartz LB. Immunoassay of tryptase from human mast cells. J Immunol Methods 1986; 86:139-42.
13. Enander I, Matsson P, Nystrand J, Andersson AS, Eklund E, Bradford TR, et al. A new radioimmunoassay for human mast cell tryptase using monoclonal antibodies. J Immunol Methods 1991; 138:39-46.
14. Vitte J. Human mast cell tryptase in biology and medicine. Mol Immunol 2015; 63:18-24.
15. Schwartz LB. Effector cells of anaphylaxis: mast cells and basophils. Novartis Found Symp 2004; 257:65-74; discussion -9, 98-100, 276-85.
16. Schwartz LB, Bradford TR, Rouse C, Irani AM, Rasp G, Van der Zwan JK, et al. Development of a new, more sensitive immunoassay for human tryptase: use in systemic anaphylaxis. J Clin Immunol 1994; 14:190-204.
17. Schwartz LB, Sakai K, Bradford TR, Ren S, Zweiman B, Worobec AS, et al. 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.
18. 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.
19. Valent P, Akin C, Arock M, Brockow K, Butterfield JH, Carter MC, et al. Definitions, criteria and global classification of mast cell disorders with special reference to mast cell activation syndromes: a consensus proposal. Int Arch Allergy Immunol 2012; 157:215-25.

20. Vitte J, Amadei L, Gouitaa M, Mezouar S, Zieleskiewicz L, Albanese J, et al. Paired acute-baseline serum tryptase levels in perioperative anaphylaxis: An observational study. Allergy 2019; 74:1157-65.
21. Borer-Reinhold M, Haeberli G, Bitzenhofer M, Jandus P, Hausmann O, Fricker M, et al. 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.
22. Phadia AB. ImmunoCAP tryptase. Directions for use. Uppsala SPA, 2008.
23. Egner W, Sargur R, Shrimpton A, York M, Green K. A 17-year experience in perioperative anaphylaxis 1998-2015: harmonizing optimal detection of mast cell mediator release. Clin Exp Allergy 2016; 46:1465-73.
24. Tacquard C, Collange O, Gomis P, Malinovsky JM, Petitpain N, Demoly P, et al. Anaesthetic hypersensitivity reactions in France between 2011 and 2012: the 10th GERAP epidemiologic survey. Acta Anaesthesiol Scand 2017; 61:290-9.
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. Laroche D, Gomis P, Gallimidi E, Malinovsky JM, Mertes PM. Diagnostic value of histamine and tryptase concentrations in severe anaphylaxis with shock or cardiac arrest during anesthesia. Anesthesiology 2014; 121:272-9.
27. Passia E, Jandus P. Using Baseline and Peak Serum Tryptase Levels to Diagnose Anaphylaxis: a Review. Clin Rev Allergy Immunol 2020; 58:366-76.
28. Egner W, Cook TM, Garcez T, Marinho S, Kemp H, Lucas DN, et al. Specialist perioperative allergy clinic services in the UK 2018: Results from the Royal College of Anaesthetists Sixth National Audit Project (NAP6) investigation of perioperative anaphylaxis. Clin Exp Allergy 2018; 48:846-61.
29. Baretto RL, Beck S, Heslegrave J, Melchior C, Mohamed O, Ekbote A, et al. Validation of international consensus equation for acute serum total tryptase in mast cell activation: A perioperative perspective. Allergy 2017; 72:2031-4.
30. De Schryver S, Halbrich M, Clarke A, La Vieille S, Eisman H, Alizadehfar R, et al. Tryptase levels in children presenting with anaphylaxis: Temporal trends and associated factors. J Allergy Clin Immunol 2016; 137:1138-42.
31. Muraro A, Roberts G, Worm M, Bilò MB, Brockow K, Fernández Rivas M, et al. Anaphylaxis: guidelines from the European Academy of Allergy and Clinical Immunology. Allergy 2014; 69:1026-45.
32. Sampson HA, Munoz-Furlong A, Campbell RL, Adkinson NF, Jr., Bock SA, Branum A, et al. Second symposium on the definition and management of anaphylaxis: summary report--Second National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network symposium. J Allergy Clin Immunol 2006; 117:391-7.
33. Ebo DG, Van Gasse AL, Decuyper, II, Uyttebroek A, Sermeus LA, Elst J, et al. Acute Management, Diagnosis, and Follow-Up of Suspected Perioperative Hypersensitivity Reactions in Flanders 2001-2018. J Allergy Clin Immunol Pract 2019; 7:2194-204.e7.
34. Sabato V, Chovanec J, Faber M, Milner JD, Ebo D, Lyons JJ. First Identification of an Inherited TPSAB1 Quintuplication in a Patient with Clonal Mast Cell Disease. J Clin Immunol 2018; 38:457-9.
35. Lyons JJ. Hereditary Alpha Tryptasemia: Genotyping and Associated Clinical Features. Immunology and allergy clinics of North America 2018; 38:483-95.
36. Sabato V, Van De Vijver E, Hagendorens M, Vrelust I, Reyniers E, Fransen E, et al. Familial hypertryptasemia with associated mast cell activation syndrome. J Allergy Clin Immunol 2014; 134:1448-50.e3.
37. Valent P, Horny HP, Escribano L, Longley BJ, Li CY, Schwartz LB, et al. Diagnostic criteria and classification of mastocytosis: a consensus proposal. Leuk Res 2001; 25:603-25.

38. Kim HY. Statistical notes for clinical researchers: Chi-squared test and Fisher's exact test. Restor Dent Endod 2017; 42:152-5.
39. Mertes PM, Laxenaire MC, Alla F. Anaphylactic and anaphylactoid reactions occurring during anesthesia in France in 1999-2000. Anesthesiology 2003; 99:536-45.
40. Mertes PM, Alla F, Tréchot P, Auroy Y, Jougla E. Anaphylaxis during anesthesia in France: an 8-year national survey. J Allergy Clin Immunol 2011; 128:366-73.
41. Garvey LH, Bech B, Mosbech H, Krøigaard M, Belhage B, Husum B, et al. Effect of general anesthesia and orthopedic surgery on serum tryptase. Anesthesiology 2010; 112:1184-9.
42. Phadia AB. ImmunoCAP tryptase. Directions for use. Uppsala SPA, 2013. 2013.
43. UK NEQAS for TryptaseEQA Scheme CPIpd.
44. Gonzalez-Quintela A, Vizcaino L, Gude F, Rey J, Meijide L, Fernandez-Merino C, et al. Factors influencing serum total tryptase concentrations in a general adult population. Clin Chem Lab Med 2010; 48:701-6.
45. Schliemann S, Seyfarth F, Hipler UC, Elsner P. Impact of age and heterophilic interference on the basal serum tryptase, a risk indication for anaphylaxis, in 1,092 dermatology patients. Acta Derm Venereol 2012; 92:484-9.
46. 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.
47. Broesby-Olsen S, Oropeza AR, Bindslev-Jensen C, Vestergaard H, Møller MB, Siebenhaar F, et al. Recognizing mastocytosis in patients with anaphylaxis: value of KIT D816V mutation analysis of peripheral blood. J Allergy Clin Immunol 2015; 135:262-4.
48. Hermans MAW, Schreurs MWJ, van Daele PLA. Systemic mastocytosis with normal serum tryptase: rule or exception? J Eur Acad Dermatol Venereol 2019; 33:e472-e4.
49. Hermans MAW, Rietveld MJA, van Laar JAM, Dalm V, Verburg M, Pasmans S, et al. Systemic mastocytosis: A cohort study on clinical characteristics of 136 patients in a large tertiary centre. Eur J Intern Med 2016; 30:25-30.
50. Sánchez-Muñoz L, Alvarez-Twose I, García-Montero AC, Teodosio C, Jara-Acevedo M, Pedreira CE, et al. Evaluation of the WHO criteria for the classification of patients with mastocytosis. Mod Pathol 2011; 24:1157-68.
51. Lyons JJ, Chovanec J, O'Connell MP, Liu Y, Šelb J, Zanotti R, et al. Heritable risk for severe anaphylaxis associated with increased α-tryptase-encoding germline copy number at TPSAB1. J Allergy Clin Immunol 2021; 147:622-32.
52. Valent P, Sperr WR, Sotlar K, Reiter A, Akin C, Gotlib J, et al. The serum tryptase test: an emerging robust biomarker in clinical hematology. Expert Rev Hematol 2014; 7:683-90.
53. Mohajeri M, Kovanen PT, Bianconi V, Pirro M, Cicero AFG, Sahebkar A. Mast cell tryptase - Marker and maker of cardiovascular diseases. Pharmacol Ther 2019; 199:91-110.
54. Pastorello EA, Farioli L, Losappio LM, Morici N, Di Biase M, Nichelatti M, et al. Serum tryptase detected during acute coronary syndrome is significantly related to the development of major adverse cardiovascular events after 2 years. Clin Mol Allergy 2015; 13:14.
55. Jesky MD, Stringer SJ, Fenton A, Ng KP, Yadav P, Ndumbo M, et al. Serum tryptase concentration and progression to end-stage renal disease. Eur J Clin Invest 2016; 46:460-74.
56. Min HK, Moxley G, Neale MC, Schwartz LB. Effect of sex and haplotype on plasma tryptase levels in healthy adults. J Allergy Clin Immunol 2004; 114:48-51.
57. Sverrild A, van der Sluis S, Kyvik KO, Garvey LH, Porsbjerg C, Backer V, et al. Genetic factors account for most of the variation in serum tryptase--a twin study. Ann Allergy Asthma Immunol 2013; 111:286-9.

Tables

Table 1. Demographics and clinical characteristics.

	Controls	POH patients					P value
		Grade 1	**Grade 2**	**Grade 3**	**Grade 4**	**All**	
Number (%)	75	19 (6)	29 (10)	171 (58)	77 (26)	296 (100)	
Age (range)	51 (19-79)	52 (22-77)	42 (3m-78)	52 (5-84)	58 (15-82)	53 (3m-84)	0.004
Sex (m/f)	45/30	8/11	14/15	53/118	39/38	114/182	0.0003
Identification of cause (%)							
Yes	-	15 (79)	14 (48)	131 (77)	65 (84)	225 (76)	0.0015
No	-	4 (21)	15 (52)	40 (23)	12 (16)	71 (24)	

Age: median (range), sex: male/female, Identification of trigger after allergic work up. Grades according to severity[8,33]. m, month

Table 2. Comparison of sensitivity (Se), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV) and accuracy (Acc) for the different tryptase decision thresholds.

Overall: POH (296) vs controls (75)					
Decision thresholds	**Se**	**Sp**	**PPV**	**NPV**	**Acc**
ΔT > 3.2 ng/mL	78	99	99,6	54	82
Consensus formula	78	95	98	52	81
%ΔT > 85% ↑	75	91	97	48	78
aST > 11.4 ng/mL	70	97	99	45	75
aST > 14 ng/mL	64	99	99	41	71

Results were generated by comparing samples from perioperative anaphylaxis patients (N = 296) and controls (N = 75). ΔT, delta tryptase; %ΔT, percentage of increase in tryptase; aST, acute serum tryptase; MCA, mast cell activation; Consensus formula, >1.2 × sBT + 2 ng/mL.

Table 3. Performance of different tryptase thresholds for different severity grades.

Overall: POH (296) vs controls (75)							
	Controls	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**	**All**	**P value**
ΔT > 3.2 ng/mL	1/75 (1%)	**7/19 (37%)**	**13/29 (45%)**	**141/171 (82%)**	**71/77 (92%)**	**232/296 (78%)**	<.001
Consensus formula	4/75 (5%)	**6/19 (32%)**	**12/29 (41%)**	**142/171 (83%)**	**71/77 (92%)**	**231/296 (78%)**	<.001
> 85% ↑	7/75 (9%)	**7/19 (37%)**	**7/29 (24%)**	**137/171 (80%)**	**72/77 (94%)**	**223/296 (75%)**	<.001
aST > 11.4 ng/mL	2/75 (3%)	**6/19 (32%)**	**8/29 (28%)**	**125/171 (73%)**	**68/77 (88%)**	**207/296 (70%)**	<.001
aST > 14 ng/mL	1/75 (1%)	2/19 (11%)	**7/29 (24%)**	**115/171 (67%)**	**66/77 (86%)**	**190/296 (64%)**	<.001

P value represents a chi square calculated for the different frequencies of the five different groups (controls, grade 1, grade 2, grade 3 and grade 4). P values between controls and severity grades separately were (controls vs grade 1, 2,3,4) were also calculated by using Chi-square test or Fisher exact test and were shown in bold font when significant. ΔT, delta tryptase; %ΔT, percentage of increase in tryptase; aST, acute serum tryptase; MCA, mast cell activation; Consensus formula, >1.2 × sBT + 2 ng/mL.

Table 4. Summary of baseline serum tryptase (bST), acute serum tryptase (aST) and delta tryptase (aST-bST) of patients with a perioperative hypersensitivity per severity grade.

	Controls	Grade 1	Grade 2	Grade 3	Grade 4	P value
Median bST (range)	2.27 (0.04-19.42)	5.20 (1.70-9.60)	4.70 (2.00-19.00)	5.00 (1.00-66.00)	5.40 (1.70-25.40)	0.47
Median aST (range)	1.92 (0.44-14.44)	7.10 (2.20-31.00)	7.40 (2.50-31.00)	20.700 (2.40-429.00)	39.10 (2.33-200)	<.001
Median delta tryptase (range)	-0.22 (-6.45 -9.90)	2.10 (-2.20-21.40)	1.50 (-2.50-43.68)	14.60 (-12.60-43.68)	29.70 (-0.97-194.71)	<.001

P value (P) represents differences between the different severity groups. Statistical difference between the median tryptase levels of the severity grades were calculated with the Kruskal-Wallis Test (P value). bST, baseline serum tryptase.

Figure legends

Figure 1. Tryptase levels for controls and perioperative hypersensitivity (POH) patients.

Control samples were acquired at induction (Tt0) and after approximately 1.5 hours (Tt1). Samples from POH were acquired +/- 60-120 minutes after onset of symptoms (aST) and at baseline (bST) at least 24 hours after onset of the reaction. Panels A and B shows the kinetics of the paired samples in patients and controls. Panels C and D show the individual data points of tryptase measurements for POH patients and controls with median and 95% confidence interval.

Figure 2. ROC curves for determination of ideal cut-off points for acute serum tryptase (aST), delta tryptase (ΔT) and percentage increase of tryptase (%ΔT).

Ideal cut-off points were established at aST ≥5.1 ng/mL, a ΔT of ≥3.2 ng/mL and %ΔT ≥85%. ΔT, delta tryptase; %ΔT, percentage of increase in tryptase.

Figure 3. Comparison of ROC curves of the different tryptase thresholds for mast cell activation.

ROC curves were made for delta tryptase (ΔT) of ≥3.2 ng/mL, percentage tryptase increase of ≥85%, the consensus formula [19], acute serum tryptase (aST)>11.4 ng/mL and aST >14 ng/mL. The performance of the different tryptase thresholds is represented by the area under the curve (AUC). Details on the statistically significant differences can be found in table E2 of the online repository. MCA, mast cell activation; consensus formula, >1.2 × sBT + 2 ng/mL.

Figure 4. Distribution of acute serum tryptase (aST) levels for controls and perioperative hypersensitivity patients.

Grades represent the severity grades. median levels and 95% confidence interval are shown for each group. The y-axis is set as a log10 scale. P values were calculated by using the Mann-Whitney U test. * Median aST level of life-threatening reactions (grade 3 and 4) were significantly higher than non-life-threatening reactions (grade 1 and 2) (p <.001).

Mast cell activation during suspected perioperative hypersensitivity: a need for paired samples analysis

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Online repository

Tables

Table E1. Causes identified after allergy work-up.

Causes							
	Controls	Grade 1	Grade 2	Grade 3	Grade 4	All grades	P value
NMBA	-	5	3	69	39	116 (39%)	0.0006
Rocuronium	-	4	3	58	35	100	
Others	-	1	0	11	4	16	
Antibiotics	-	2	2	30	8	42 (14%)	0.42
Cefazolin	-	2	2	25	7	36	
Others	-	0	0	5	2	6	
Chlorhexidine	-	2	1	13	7	23 (8%)	0.70
Latex	-	3	4	10	2	19 (6%)	0.07
Others	-	3	4	9	9	25 (8%)	0.19
Unknown	-	4	15	40	12	71 (24%)	0.004
Total	-	19	29	171	77	296	

Table E2. Comparison of the different tryptase thresholds by comparing the AUC of the different thresholds.

Decision threshold 1	AUC 1	Decision threshold 2	AUC 2	P - value
ΔT of ≥3.2 ng/mL	0.89	Tryptase increase of 85%	0.83	0.002
ΔT of ≥3.2 ng/mL	0.89	aST > 11.4 ng/mL	0.84	<.001
ΔT of ≥3.2 ng/mL	0.89	aST > 14 ng/mL	0.81	<.001
ΔT of ≥3.2 ng/mL	0.89	Consensus formula	0.86	0.08
Tryptase increase of 85%	0.83	aST > 11.4 ng/mL	0.84	0.75
Tryptase increase of 85%	0.83	aST > 14 ng/mL	0.81	0.46
Tryptase increase of 85%	0.83	Consensus formula	0.86	0.04
aST > 11.4 ng/mL	0.84	aST > 14 ng/mL	0.81	0.02
aST > 11.4 ng/mL	0.84	Consensus formula	0.86	0.09
aST > 14 ng/mL	0.81	Consensus formula	0.86	0.007

Difference in AUC was determined by calculating chi square (p value). ΔT, delta tryptase; %ΔT, percentage of increase in tryptase; aST, acute serum tryptase; MCA, mast cell activation; Consensus formula, $>1.2 \times sBT + 2$ ng/mL.