

ORIGINAL ARTICLE

Drug Allergy, Insect Sting Allergy, and Anaphylaxis

Paired acute-baseline serum tryptase levels in perioperative anaphylaxis: An observational study

Joana Vitte¹  | Laurent Amadei² | Marion Gouitaa³ | Soraya Mezouar¹ | Laurent Zieleskiewicz² | Jacques Albanese⁴ | Nicolas Bruder⁵ | David Lagier⁵ | Paul M. Mertès⁶ | Jean-Louis Mège¹ | Lawrence B. Schwartz⁷ | Marc Leone^{1,2}

¹Aix-Marseille Univ, IRD, APHM, MEPHI, IHU Méditerranée Infection, Marseille, France

²Aix-Marseille Univ, APHM, Hôpital Nord, Marseille, France

³Aix-Marseille Univ, APHM, Hôpital Nord, Service de Pneumologie, Marseille, France

⁴Aix-Marseille Univ, APHM, Hôpital de la Conception, Service d'Anesthésie et de Réanimation, Marseille, France

⁵Aix-Marseille Univ, APHM, Hôpital de la Timone, Service d'Anesthésie et de Réanimation, Marseille, France

⁶Strasbourg Univ, HUS, Nouvel Hôpital Civil, FMTS, Service d'Anesthésie Réanimation, Strasbourg, France

⁷Division of Rheumatology, Allergy and Immunology, Virginia Commonwealth University, Richmond, Virginia, VA, USA

Correspondence

Joana Vitte, IHU Méditerranée Infection, UF Immunologie-Allergologie, Marseille, France.
Email: jvitte@ap-hm.fr

Abstract

Background: Anaphylaxis is recognized mainly through clinical criteria, which may lack specificity or relevance in the perioperative setting. The transient increase in serum tryptase has been proposed since 1989 as a diagnostic tool. Sampling for well-defined acute and baseline determinations has been recommended. We assessed the performance of four proposed algorithms with tightly controlled time frames for tryptase sampling, their robustness with inadequate sampling times, and the possible use of mature tryptase determination.

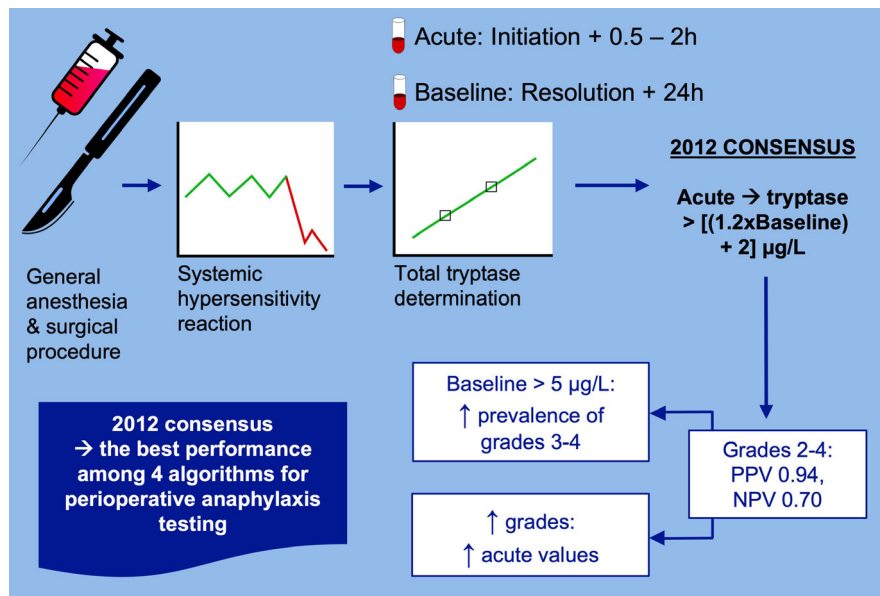
Methods: A retrospective study was performed on 102 adult patients from the Aix-Marseille University Hospitals who had experienced a perioperative hypersensitivity reaction clinically suggesting anaphylaxis. EAACI and ICON criteria were used to diagnose anaphylaxis. Mature and total serum tryptase levels were measured.

Results: Based on EAACI guidelines, clinical diagnostic criteria for anaphylaxis were found in 76 patients and lacking in 26. The most effective algorithm was the international consensus recommendation of 2012 that acute total tryptase levels should be greater than $([1.2 \times \text{baseline tryptase}] + 2) \mu\text{g/L}$ to be considered a clinically significant rise. In our cohort, this algorithm achieved 94% positive predictive value (PPV), 53% negative predictive value (NPV), 75% sensitivity, 86% specificity, and a Youden's index value of 0.61. A detectable acute mature tryptase level showed lower sensitivity, particularly in patients with acute total tryptase levels lower than $16 \mu\text{g/L}$. Acute tryptase levels varied as a function of the clinical severity of anaphylaxis.

Conclusion: Total tryptase levels in serum discriminated between nonanaphylactic and anaphylactic events in a perioperative setting when acute and baseline levels were collected and analyzed by the consensus algorithm.

KEYWORDS

acute tryptase, anaphylaxis, anesthesia, baseline tryptase, hypersensitivity reaction, mast cell, mature tryptase, perioperative anaphylaxis, positive predictive value



GRAPHICAL ABSTRACT

The 2012 consensus that acute tryptase should be greater than $[(1.2 \times \text{baseline tryptase}) + 2] \mu\text{g/L}$ performs best among four algorithms for tryptase interpretation during perioperative anaphylaxis. The 2012 consensus that acute tryptase should be greater than $[(1.2 \times \text{baseline tryptase}) + 2] \mu\text{g/L}$ performs best for reaction grades 2, 3 and 4. Higher anaphylaxis grades are associated with higher levels of acute tryptase and more prevalent if baseline tryptase levels are above $5 \mu\text{g/L}$.

PPV: Positive predictive value; NPV: Negative predictive value

1 | INTRODUCTION

Hypersensitivity reactions account for 9%-19% of complications related to perioperative anesthesia.¹⁻³ The latest World Allergy Organization (WAO) definition of anaphylaxis is "a life-threatening systemic or hypersensitivity reaction (SHR) with sudden onset".⁴ The estimated incidence of perioperative anaphylaxis (PA), that is, anaphylaxis during the perioperative period,¹ is 1 in 20 000 to 1 in 10 000,²⁻⁵ although underdiagnosis might be frequent.⁴ Despite recent progress, the diagnosis of PA remains challenging.^{1,6} Anaphylaxis is recognized mainly through clinical criteria.^{4,7} As an example, EAACI criteria for anaphylaxis include "reduced blood pressure after exposure to a known allergen for that patient",⁷ but previous reactions to drugs or intake of pholcodin may not be reported prior to the surgery. Reactions may also occur upon the first exposure. During the perioperative setting, as opposed to a usual workup of immediate SHR, the question is not simply whether allergic symptoms meet criteria for anaphylaxis, but whether the acute worsening of a patient's status stems from an immediate hypersensitivity cause. Laboratory results supporting mast cell (MC) degranulation are valuable for classifying a perioperative event as anaphylaxis, even if they are not available until after the acute event subsides.⁸ The transient increase in serum tryptase has been proposed since 1989 as a diagnostic tool for anaphylaxis⁹ and sampling for acute and baseline determination has been recommended,^{4,5,7-10} as well as a consensus algorithm for determining what is a clinically significant rise in the acute above the baseline level of tryptase.¹⁰ The WAO and the consensus of the 2010 Working Conference on Mast Cell Disorders

recommend tryptase determination acutely, 15 minutes to 3 hours after the onset of symptoms, and at baseline, either before or at least 24 hours after complete resolution of all symptoms and signs.^{4,10}

Tryptase comprises a family of MC specific proteases.⁹ The α and β isoforms contribute to tryptase blood concentrations, at baseline as protryptases and with systemic anaphylaxis as pro- plus mature tryptases.^{11,12} A portion of the α and β monomeric pro-forms are secreted continuously at baseline from MC at rest. Another portion of the pro-forms are converted inside MC to mature tryptase tetramers that are stored in secretory granules, and are secreted when MC are activated to degranulate. The collective measurement of the pro- and mature forms of α and β tryptases provides the total serum tryptase value and will be called tryptase in this manuscript, whereas measurements of mature α and β tryptases will be called mature tryptase. An acute serum tryptase (sAT) level greater than $\{[1.2 \times \text{serum baseline tryptase (sBT)}] + 2\} \mu\text{g/L}$ has been recommended as a consensus decision threshold to confirm clinically significant MC degranulation.¹⁰ Other decision thresholds for total tryptase variation during MC degranulation have been proposed, including sAT being $> (1.35 \times \text{sBT})$,¹³ $> (1.2 \times \text{sBT})$, $> (3 + \text{sBT}) \mu\text{g/L}$,¹⁴ $> 11.4 \mu\text{g/L}$ (the manufacturer's upper limit of reference values for sBT,¹⁵) or $> 14 \mu\text{g/L}$.¹⁶ Yet, serial tryptase determination is seldom implemented and a common decision threshold has not been adopted in daily practice.^{14,16-18}

Our main objective was to determine the diagnostic performance of paired sAT and sBT at adequate sampling times for the diagnosis of PA in adults. Secondary objectives included evaluating the impact of inadequate tryptase sample timing on the performance of

algorithms, evaluating the diagnostic value of mature tryptase, and searching for a link between sAT, sBT, and severity of PA.

2 | PATIENTS AND METHODS

2.1 | Study population

Patients from the Aix-Marseille University Hospitals (Hôpital Nord, Hôpital de la Timone, Hôpital de la Conception), Marseille, France, aged 18 years or more, having experienced a perioperative SHR clinically suggesting anaphylaxis from November 2011 through May 2017, and for whom at least two tryptase determinations had been performed, were retrospectively identified.

2.2 | Ethics statement

This retrospective, noninterventional study was based on the review of clinical and laboratory medical records. Under the French law, ethics committee approval and patient consent were not required for this type of noninterventional study, provided the patients had received information about the potential use of anonymized medical data for research purposes, and retained the right to oppose it (SFAR CERAR committee IRB 00010254-2018-004; CIL/APHM registry 2017-24).

2.3 | Diagnosis of anaphylaxis

According to current international guidelines,^{4,7,19-21} diagnosis of anaphylaxis was based on clinical criteria aiming at the recognition of “a serious, life-threatening generalized or systemic hypersensitivity reaction”²⁰ “with sudden onset”.⁴ Following the ICON guidelines, the term “anaphylaxis” was preferred to “anaphylactic shock” because shock is not necessarily present in patients with anaphylaxis.¹⁹ Conversely, mild reactions graded 1 or 2 are not consensually considered as anaphylaxis.^{1,6} Confirmation and grading of the SHR were performed retrospectively, after the acute event, either by the anesthesiology team while the patient was in the hospital or by an allergist, 6 weeks to 3 months after the acute event. In both cases, results of laboratory tests for confirmation of clinical diagnosis, that is, tryptase results, together with IgE results for suspected triggers, were available for the medical team at the retrospective diagnostic evaluation. Specialized allergy workup was performed by the same allergist (MG) and relied on clinical information and a set of complementary procedures. Allergy skin tests, including skin prick tests and intradermal tests, serum specific immunoglobulin (Ig) E, and drug challenges, were employed in line with current recommendations.^{5,21} A specialized allergy workup was performed on 44 patients. The etiological diagnosis was based on history, timing, and in vitro tests only, in 12 patients.

2.4 | Data collection

For each patient, we collected data on age, gender, atopic history (asthma, allergic rhinitis, atopic dermatitis, food, drug, or latex allergy), operative procedures, and anesthesia records. Perioperative

involvement of organs, including cutaneous, gastrointestinal, respiratory, and cardiovascular systems, was documented. The severity score was graded using the 4-class scale adapted from Ring and Messmer.²² The preoperative physical status was evaluated with the American Society of Anesthesiology physical status (ASA-PS) classification describing patient demographics, clinical characteristics, and comorbidities, widely used by anesthesiologists as a risk predictor.^{23,24}

2.5 | Laboratory workup

Serum total tryptase and specific IgE were measured with ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden). IgE to suspect molecules received during the perioperative procedure prior to the initiation of symptoms were routinely assayed in the perioperative samples: latex, penicillins G and V, amoxicillin, cefaclor, succinylcholine, quaternary ammonium groups. The lower limit of quantification was 1 µg/L for tryptase and 0.10 kU_A/L for IgE.

2.6 | Samples

For perioperative SHR events clinically suggesting anaphylaxis, our institution recommends two sampling time-points: an acute sample collected 0.5-2 hours after the initiation of symptoms and a baseline sample before the start of a reaction or at least 24 hours after resolution of all signs and symptoms.¹⁰ Blood samples are sent to the laboratory with a document specifying the exact time of sampling, the exact time of symptom initiation, the complete list of drugs the patient received prior to the initiation of symptoms, and whether latex equipment was employed.

2.7 | Mature tryptase determination

Mature tryptase was measured in the VCU Laboratory using the biotin-conjugated G5 monoclonal anti-tryptase IgG for detection and the B12 monoclonal anti-tryptase IgG for capture.^{9,12,25} Consecutive acute samples from 42 patients were retrospectively selected from the study population. The selection was blinded with respect to the final confirmation or rejection of PA. Samples belonged to three groups based on sAT level: ≤11.4 µg/L (n = 35), 12-20 µg/L (n = 4), and >20 µg/mL (n = 3). In 13 of these 42 patients, mature tryptase was also assayed in baseline samples.

2.8 | Statistical analysis

Category variables were presented as frequencies. Continuous quantitative variables were each presented as a median, range, and interquartile range (IQR). The association between category variables was evaluated with the khi-2 test and Fisher's exact test as applicable. Continuous variables were compared with Student's *t* test and multiple *t* tests for comparison of groups. PPV, NPV, sensitivity, specificity, and Youden's index of tryptase concentrations were calculated, with sensitivity + specificity – 1 = Youden's index. The

TABLE 1 Relevant demographic and clinical features according to the final diagnosis of perioperative anaphylaxis (PA) and the severity score. Patient groups are presented according to the final diagnosis of confirmed or rejected PA and severity scoring according to Ring and Messmer. Briefly, grade 1, cutaneous-mucous signs; grade 2, previous plus cardiovascular and/or respiratory signs; grade 3, previous + cardiovascular shock ± bronchospasm; grade 4, previous ± cardiac arrest. Significant differences between PA grades and nonanaphylactic events are presented in bold characters. IQR, interquartile range; n, sample size; P, P-value (multivariate analysis); PA, perioperative anaphylaxis

	No PA n = 26	Grade 1 n = 9	Grade 2 n = 12	Grade 3 n = 45	Grade 4 n = 10	All PA n = 76	P
Age (years) median, IQR	57 (44-67)	37 (28-50)	47 (34-58)	55 (37-67)	64 (62-79)	53 (37-66)	0.009
Range	18-88	23-88	21-70	21-83	56-86	21-88	
Adequate tryptase sampling	81% (21/26)	89% (8/9)	75% (9/12)	84% (38/45)	90% (9/10)	84% (64/76)	0.9
Atopy	38% (10/26)	56% (5/9)	42% (5/12)	40% (18/45)	20% (2/10)	39% (30/76)	0.6
Skin involvement	15% (4/26)	100% (9/9)	83% (10/12)	60% (27/45)	20% (2/10)	63% (48/76)	2 × 10⁻⁶
Respiratory involvement	38% (10/26)	0/9	33% (4/12)	44% (20/45)	20% (2/10)	34% (26/76)	0.1
Gastrointestinal involvement	8% (2/26)	0/9	0/12	4% (2/45)	0/10	2% (2/76)	0.3
Cardiovascular involvement	58% (15/26)	0/9	84% (10/12)	93% (42/45)	100% (10/10)	82% (62/76)	1 × 10⁻⁸
Allergy workup	23% (6/26)	44% (4/9)	67% (8/12)	51% (23/45)	40% (4/10)	51% (39/76)	0.01

associations between sAT, sBT, tryptase acute-to-baseline difference, and clinical severity were evaluated with Spearman's rank test. Statistical analysis was performed with GraphPad Prism version 7.

3 | RESULTS

3.1 | Demography and clinical presentation of PA

We identified 102 patients with perioperative SHR clinically suggesting PA, for whom at least two serum tryptase determinations had been performed from November 2011 through May 2017 (Figure S1). According to the EAACI clinical criteria,⁷ PA was confirmed in 76 (75%) patients. PA was associated with an increased prevalence of cutaneous and cardiovascular symptoms, and more frequent referral to an allergist, positive allergy skin tests, and specific ImmunoCAP IgE positivity (Table 1). Respiratory and gastrointestinal symptoms, atopic history, and ASA-PS score for preoperative physical status did not differ between anaphylactic and nonanaphylactic events. Cutaneous symptoms were more frequent during grade 1 and 2 PA, while cardiovascular symptoms and older age were preferentially associated with grade 4 PA. No perioperative death occurred in the study sample.

3.2 | Allergy workup

Forty-four (43%) patients, comprising 38 with clinically confirmed PA and 6 without, attended the allergy department after the perioperative event. Detailed results of allergy workup are presented in Material S1.

3.3 | Time frame and number of time-points for tryptase determination

The patients were categorized as follows: adequate sAT and sBT (n = 85), adequate sAT and inadequate sBT (n = 16), inadequate sAT and adequate sBT (n = 1).

Paired sAT and sBT determinations within the recommended time frame occurred in 85 (83%) patients (Figure 1). Proper sAT but incorrect sBT timing was found in 16 (16%) patients: 14 because the "baseline" sample was taken 4-20 hours after the onset of the SHR, and two because blood was taken less than 15 minutes after the initiation of SHR symptoms, followed by an optimal sample for sAT being taken between 30 minutes and 2 hours after onset of symptoms (Figure 1).

In 10 out of 16 patients with a proper sAT but inadequate sBT (9 taken 4-20 hours after the initiation of symptoms, 1 taken <15 minutes after), tryptase algorithms confirmed a transient increase in tryptase concentrations during the perioperative event. Overall, interpretation of tryptase results was feasible in 85+10 = 95 (93%) patients (Figure 1).

3.4 | Diagnostic performance of tryptase algorithms for PA

The diagnostic performances of three published algorithms and of the manufacturer's upper reference value were compared. One patient for whom sAT sampling had not been performed was excluded from the analysis.

In 85 patients (64/85, 75% confirmed PA) with adequate sAT and sBT sampling times, the highest Youden's index (0.61) was obtained with $[(1.2 \times \text{sBT}) + 2] \mu\text{g/L}$. The other algorithms displayed lower Youden's index due to either lower sensitivity (the manufacturer's 11.4 $\mu\text{g/L}$ limit and the $[\text{sBT} + 3] \mu\text{g/L}$ algorithm), or lower specificity (the $[1.35 \times \text{sBT}] \mu\text{g/L}$ algorithm) (Table 2).

Among 16 patients with inadequate sBT timing, 12 (75%) were confirmed as PA, and in 10 of these 12 a transient increase in sAT was observed, resulting in Youden's index ranging from 0.58 with the manufacturer's 11.4 $\mu\text{g/L}$ threshold to 0.83 with the three other

FIGURE 1 Definition of patient groups according to tryptase sampling times. Adequate acute tryptase (sAT) was defined as a sample taken 0.5–2 h after the initiation of symptoms of a perioperative hypersensitivity reaction clinically suggesting anaphylaxis. Adequate baseline tryptase (sBT) was defined as a sample taken ≥ 24 h after complete resolution of signs and symptoms of hypersensitivity. Intermediate samples were defined as taken more than 3 hours, but less than 24 hours, after the initiation of symptoms. Samples taken less than 15 min after the initiation of symptoms were not considered as acute tryptase, given the risk of false negative results. Overall, tryptase changes during the perioperative event could be interpreted in 95 patients (denoted in bold font), of whom 85 had had adequate acute and baseline tryptase determination

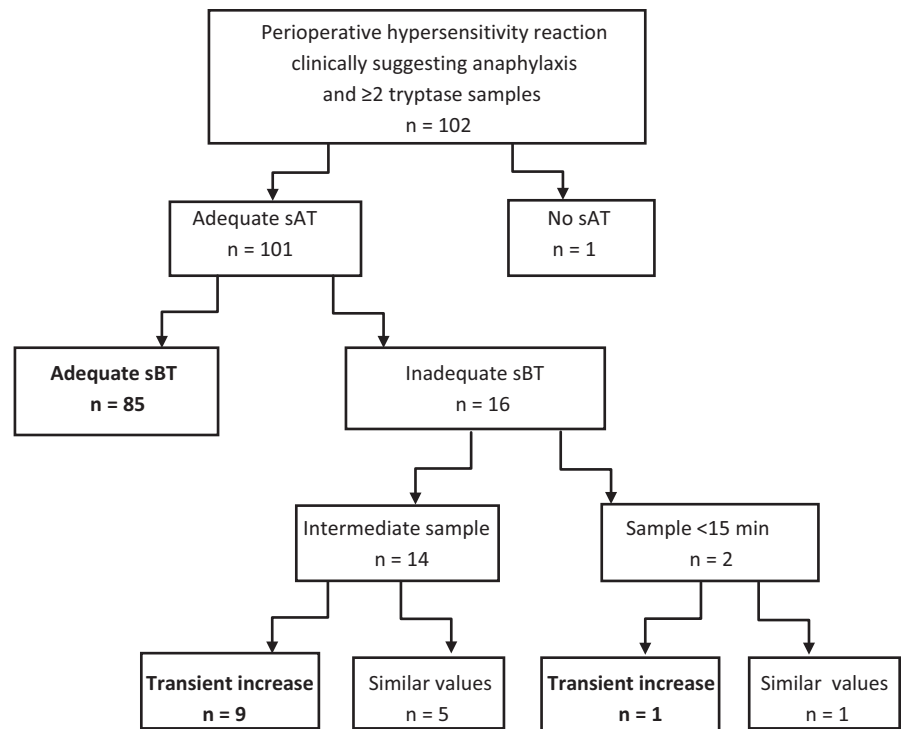


TABLE 2 Compared diagnostic performances of tryptase algorithms for perioperative anaphylaxis (PA). Adequate timing (n = 85) denoted paired acute tryptase sampling 0.5–2 h after the initiation of hypersensitivity symptoms suggesting PA, and baseline tryptase ≥ 24 h after complete resolution of symptoms and signs of anaphylaxis. Inadequate timing (n = 16) denoted paired tryptase sampling with an adequate acute sample and an inadequate baseline tryptase sample less than 24 h after symptom resolution. Confirmed PA was 75% in both adequate (64/85) and inadequate (12/16) timing groups. One patient lacking acute tryptase determination was excluded from the analysis. IQR, interquartile range; NPV, negative predictive value; PA, perioperative anaphylaxis; PPV, positive predictive value; Se, sensitivity; Sp, specificity; Y, Youden's index

Algorithm	Adequate timing (n = 85)					Inadequate timing (n = 16)					Overall (n = 101)				
	Se	Sp	PPV	NPV	Y	Se	Sp	PPV	NPV	Y	Se	Sp	PPV	NPV	Y
$>1.35 \times \text{sBT}$	0.81	0.52	0.84	0.48	0.33	0.83	1	1	0.66	0.83	0.82	0.60	0.86	0.52	0.42
$>1.2 \times \text{sBT} + 2 \mu\text{g/L}$	0.75	0.86	0.94	0.53	0.61	0.83	1	1	0.66	0.83	0.76	0.88	0.95	0.55	0.64
$>\text{sBT} + 3 \mu\text{g/L}$	0.72	0.86	0.94	0.50	0.58	0.83	1	1	0.66	0.83	0.74	0.88	0.95	0.52	0.62
$>11.4 \mu\text{g/L}$	0.53	0.95	0.97	0.40	0.48	0.83	0.75	0.91	0.60	0.58	0.58	0.92	0.96	0.42	0.50

algorithms (Table 2). Tryptase was uninterpretable in 6 (38%) out of 16 inadequate pairs.

The prevalence and severity of PA were similar in patients with and without adequate sampling times (not shown). sAT was higher in patients with inadequate sampling times (median 22.8 $\mu\text{g/L}$, IQR 6.1–42.1) vs patients with adequate sampling times (median 9.0 $\mu\text{g/L}$, range 3.9–21.9), although the difference was not significant (P 0.15, not shown).

3.5 | Acute and baseline tryptase concentrations and clinical severity of PA

sAT levels, analyzed in the overall SHR population (n = 101), were higher in patients with grade 2, 3, and 4 PA as compared to the nonanaphylactic ones (Figure 2). The difference between sAT and

sBT levels, analyzed in patients with adequate sampling times (n = 85), was higher in patients with grade 2–4 PA, consistent with higher sensitivity of tryptase algorithms (Table 3).

Levels of sBT $>5 \mu\text{g/L}$ were more prevalent in the patients experiencing grade 3 and 4 PA (Table 3).

sAT was higher than sBT in 15 out of 21 (71%) nonanaphylactic events with adequate sAT and sBT. Although the increase did not exceed 236% of sBT, it resulted in 3/15 (20%) false positives with the $(1.2 \times \text{sBT}) + 2 \mu\text{g/L}$ and the $(\text{sBT} + 3) \mu\text{g/L}$ algorithms, and in 11/15 (73%) false positives with the $(1.35 \times \text{sBT}) \mu\text{g/L}$ algorithm (not shown).

3.6 | Mature tryptase determination

Mature tryptase was detected in 6/42 acute samples. Detectable mature tryptase was associated with grade 3 and 4 PA, with higher

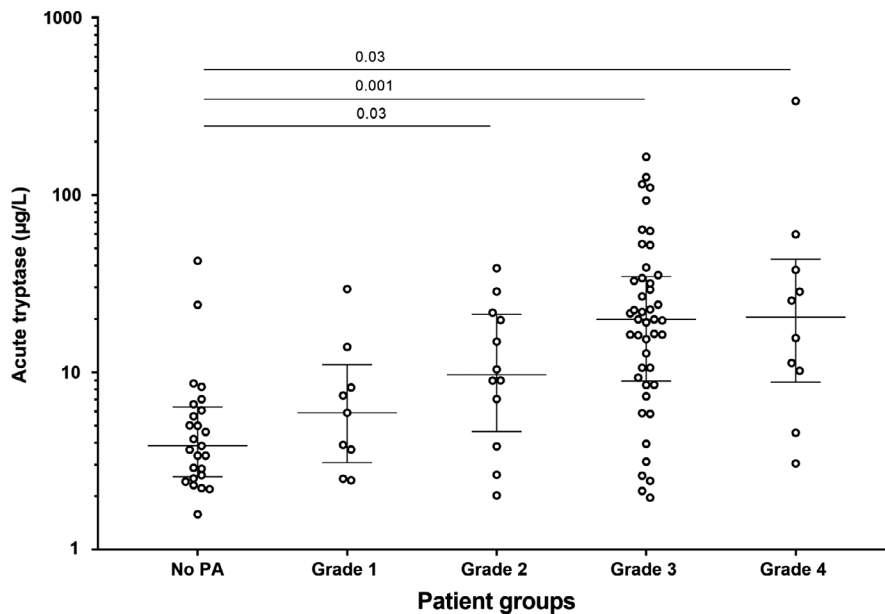


FIGURE 2 Relationship between acute tryptase values and perioperative anaphylaxis diagnosis and severity. Data are presented for the 101 patients with available acute tryptase determination. Median acute tryptase with IQR is shown for each patient group. Tryptase values are presented as raw data ($\mu\text{g/L}$). The y-axis is set as a log10 scale. IQR, interquartile range; P, P-value. Results were considered as significant when $P < 0.05$

TABLE 3 Tryptase values and algorithm performance according to perioperative anaphylaxis (PA) grades. Patient groups are presented according to the final diagnosis (clinically established PA or no PA) and severity scoring according to Ring and Messmer. All acute ($n = 101$) and baseline ($n = 102$) tryptase results are presented. Tryptase difference acute-to-baseline calculations are restricted to 85 patients with adequate sampling times for acute and baseline tryptase, that is, acute tryptase 0.5–2 hours after the initiation of symptoms suggesting anaphylaxis and baseline tryptase ≥ 24 h after complete resolution of symptoms and signs of anaphylaxis. Significant differences between anaphylaxis and nonanaphylaxis groups are shown in bold font. IQR, interquartile range; n, sample size; PA, perioperative anaphylaxis; sBT, serum baseline tryptase determination; P, P-value

	No PA	PA					Multiple t test (P)	Correlation Spearman's r; 95% confidence interval; P
		Grade 1	Grade 2	Grade 3	Grade 4	All		
Acute tryptase higher than:								
1.35 × sBT	48% (10/21)	25% (2/8)	89% (8/9)	92% (35/38)	78% (7/9)	81% (52/64)	7 × 10 ^{−5}	
(1.2 × sBT) + 2 µg/L	14% (3/21)	13% (1/8)	67% (6/9)	92% (35/38)	67% (6/9)	75% (48/64)	2 × 10 ^{−8}	
sBT + 3 µg/L	14% (3/21)	13% (1/8)	67% (6/9)	87% (33/38)	67% (6/9)	72% (46/64)	3 × 10 ^{−7}	
11.4 µg/L	8% (2/25)	22% (2/9)	42% (5/12)	69% (31/45)	60% (6/10)	58% (44/76)	2 × 10 ^{−5}	0.48 (0.30-0.62; 0.0001)
Tryptase difference acute-to-baseline (%)								
N	21	8	9	38	9	64		0.36 (0.16-0.53; 0.0008)
Median	134	119	280	440	354	359	2 × 10 ^{−16}	
IQR	98-148	104-144	200-434	242-532	161-506	183-502		
Range	71-236	81-751	122-498	52-1631	89-4934	52-4934		
Baseline tryptase >5 µg/L	18% (4/22)	25% (2/8)	0/9	45% (17/38)	56% (5/9)	38% (24/64)	0.02	

sAT and higher difference between sAT and sBT levels (Table 4). In fact, mature tryptase was not detected in samples with total sAT level lower than 16 $\mu\text{g/L}$, irrespective of the clinical severity (Table S1). Mature tryptase levels did not identify any of 12 patients with grade 1 and 2 PA and missed 10/15 patients with grade 3 PA and 4/5 of those with grade 4 PA. In acute samples with detectable mature tryptase, its level accounted for only a fraction of the total tryptase level (Table S1).

4 | DISCUSSION

We set out to assess the value of proposed algorithms for tryptase interpretation when adequate sampling for acute and baseline total tryptase is performed, their robustness in case of inadequate sampling times, and the value of mature tryptase determination in a real-life, retrospective study on PA. We included 102 patients who had experienced a perioperative SHR clinically suggesting anaphylaxis and who

TABLE 4 Comparison of patients with and without detectable acute mature tryptase. Mature tryptase was measured using the biotin-conjugated G5 monoclonal anti-tryptase IgG for detection and the B12 monoclonal anti-tryptase IgG for capture in the VCU Laboratory (Pr LB Schwartz) in acute samples from 42 patients who had experienced a perioperative hypersensitivity reaction clinically suggesting anaphylaxis. Patients were retrospectively selected and divided into those with low levels of acute tryptase (≤ 11.4 $\mu\text{g/L}$ [$n = 35$]), those with moderate levels of acute tryptase (12–20 $\mu\text{g/L}$ [$n = 4$]), and those with high acute tryptase levels (37, 39 and 93 $\mu\text{g/L}$ [$n = 3$]). The selection was blinded with respect to the final confirmation or rejection of perioperative anaphylaxis. Significant differences associated with the detection of mature tryptase in acute samples are shown in bold font ($P \leq 0.03$). F, female; IQR, interquartile range; M, male; SHR, systemic hypersensitivity reaction

	Detectable acute mature tryptase	Undetectable acute mature tryptase
Sample size	6	36
Age (years)	57 (41–61)	61 (41–72)
Median, IQR range	34–66	21–88
Sex ratio (M/F)	6 F, no male	14 M, 22 F
Storage time (years)		
Median, IQR	2.0 (0.8–3.2)	2.0 (0.8–3.7)
Range	0–4	0–5
Mature tryptase ($\mu\text{g/L}$)		
Median, IQR	3.5 (1.7–7.8)	<1
Range	1–20	
Acute total tryptase ($\mu\text{g/L}$)		
Median, IQR	27.2 (16.4–38.7)	6.0 (3.0–9.0)
Range	16–93	2–13
Total net tryptase variation (acute-baseline [$\mu\text{g/L}$])		
Median, IQR	21.5 (13.2–29.9)	2.5 (0.7–5.1)
Range	12–85	0–10
Baseline tryptase ($\mu\text{g/L}$)		
Median, IQR	6.0 (3.5–8.0)	3.4 (2.5–4.2)
Range	3.2–9.0	1–10
Confirmed mast cell degranulation according to the $(1.2 \times \text{sBT} + 2)$ $\mu\text{g/L}$ algorithm	100% (6/6)	42% (15/36)
SHR grade		
Median, IQR	3 (3–3)	2 (0–3)
Range	3–4	0–4
Detail of SHR severity	5 grade 3; 1 grade 4	10 no anaphylaxis; 4 grade 1; 8 grade 2; 10 grade 3; 4 grade 4

had had at least two sequential blood samples collected for tryptase level determination. Based on clinical criteria from EAACI and ICON guidelines,^{4,7,19,20} PA was rejected in 26 and confirmed in 76 of these

patients. Adequate tryptase sampling for sAT (0.5–2 hours after the initiation of symptoms) was performed in almost all patients (101/102), while for sBT (≥ 24 h after the complete resolution of symptoms and signs of anaphylaxis), the figures were lower (86/102, 84%). Overall, paired adequate sAT and sBT values were available in 85 patients.

The diagnostic performances of three algorithms were compared. The most effective was the international consensus recommendation of 2012¹⁰ that sAT should be greater than $([1.2 \times \text{sBT}] + 2)$ $\mu\text{g/L}$ to be considered a clinically significant rise. This algorithm achieved 0.94 PPV, 0.53 NPV, 0.75 sensitivity, 0.86 specificity, and a Youden's index value of 0.61 in cases of adequate timing of sAT and sBT sampling. This compound algorithm takes into account sBT and its absolute elevation, achieving good sensitivity even when sAT is within the normal range of 1–11.4 $\mu\text{g/L}$, and excellent specificity through correct classification of nonanaphylactic patients. This algorithm performed poorly in patients with grade 1 reactions, that is, its sensitivity increased with clinical severity, particularly as it related to a drop in blood pressure. Issues related to specificity likely include the small increases in sAT concentrations occurring with random variations or nonanaphylactic events, and mechanisms of anaphylaxis that do not involve MC degranulation.^{4,26} Indeed, MC activation has been reported in atherosclerosis-related cardiovascular events,^{27,28} coagulation abnormalities,^{29,30} and response to infectious agents.^{31,32} In line with previous cohorts,^{14,16–18,33} our results confirm that the consensus algorithm $\text{sAT} > ([1.2 \times \text{sBT}] + 2)$ $\mu\text{g/L}$ is a good compromise between empirical thresholds such as $> (1.35 \times \text{sBT})$, which privileged sensitivity as high as 0.91 in previous literature^{12,34} and 0.81 in our study, or $(\text{sBT} + 3)$ $\mu\text{g/L}$, which resulted in sensitivity values as low as 0.63 in the literature^{33,34} and 0.72 in our hands. It achieves a good performance level even in patients whose sAT does not exceed 11.4 $\mu\text{g/L}$. In our cohort, these patients accounted for 42% of confirmed PA, while the NAR-COS (National Adverse Reactions Advisory Service, Sheffield) cohort of UK perioperative events reported 54% out of 3400, 1998–2015.¹⁴ This underlines the major pitfall of dismissing MC degranulation in the absence of an elevation of sAT above 11.4 $\mu\text{g/L}$.

Mature tryptase was measured in a subset of 42 patients with an in-house method.²⁵ Mature tryptase consists of both tetramers that are functionally active molecules stored in the secretory granules of MC, and monomers that either never formed tetramers or decayed into inactive monomers from their tetramers.^{11,12} A serum level ≥ 1 $\mu\text{g/L}$ likely reflects MC degranulation releasing preformed mature tryptase tetramers. To our surprise, mature tryptase was only detected in acute samples with a total tryptase level of 16 $\mu\text{g/L}$ or higher, and it only accounted for a minor part of total tryptase level, reflecting both mature tryptase and protryptases. The acute rise over baseline in total tryptase during mild to moderate episodes of anaphylaxis in the absence of a detectable rise in mature tryptase has been reported previously.^{25,35} Overall, mature tryptase determinations did not improve diagnostic sensitivity for the identification of MC degranulation in patients with $\text{sAT} < 16$ $\mu\text{g/L}$. Thus, the diagnostic sensitivity of an acute mature tryptase level is less than that of acute and baseline total tryptase levels when analyzed by the consensus algorithm. Only in cases where an acute sample alone is available might the

mature tryptase level by itself or together with a total tryptase level be more helpful than the total tryptase level alone.

We found an association between higher sAT, higher acute-to-baseline level variations, and grade 3 and 4 PA. This result is in line with the NARCOS findings,¹⁴ in contrast to some earlier studies.³⁶ We speculate that a tight control of proper timing for sAT and sBT sampling is of paramount importance. In patients with confirmed PA, sBT levels >5 µg/L were more prevalent in grade 3 and 4 reactions. Elevated sBT may indicate MC-related conditions such as mastocytosis or hereditary alpha-tryptasemia,^{37,38} and has been associated in some but not all studies with an increased risk of PA.^{39,40}

Tryptase determination at the appropriate time-points is not always achieved in clinical practice.^{14,17,18} In our study, sAT measured between 0.5 and 2 hours after the onset of SHR symptoms was available in 99% patients, but optimal sBT samples were lacking in 16%. Nevertheless, our results showed the robustness of the compound algorithm in real-life, as 63% (10/16) of the latter allowed a confirmatory result for clinically significant MC degranulation when used as sBT. This result is not unexpected given that the peak net tryptase level, occurring 0.5–1.5 hours after insect sting-triggered anaphylaxis, declines with a half-life of about 2 hours.⁹ Sensitivity (0.83), specificity,¹ PPV,¹ and Youden's index (0.83) were higher with the consensus algorithm as well as with the other algorithms in the group lacking sBT. As sAT was higher too, we speculate that the knowledge of an elevated sAT might have been perceived by anesthesiologists as a confirmation of PA even in the absence of comparison with sBT, especially with grades 2 to 4, hence making sBT sampling seem dispensable.

Postanaphylaxis management recommendations include referral to an allergist.^{7,18} In our cohort, 43% of patients with a perioperative SHR clinically suggesting anaphylaxis were subsequently seen by an allergist. This figure is stable over decades, despite continued efforts for medical education.^{41,42} The most frequent culprits were NMBA, antibiotics, and latex in 18 (28%), 13 (20%), and 9 (14%) of the anaphylactic patients. Considering patients with adequate sAT and sBT sampling times, the consensus algorithm confirmed perioperative MC degranulation in 17/17 NMBA cases, in 12/13 antibiotic cases, and in 6/7 latex cases.

In our study, nonanaphylactic patients often displayed severe features, such as cardiovascular and respiratory involvement, which can be due to both anaphylactic and nonanaphylactic causes. Current EAACI and ICON guidelines state that anaphylaxis, including PA, is a clinical diagnosis, while laboratory tests only have a confirmatory value. Our real-life study showed that tryptase determination in the perioperative setting has become a routine procedure and therefore can be a reliable supplementary criterion for PA. Tryptase levels clearly discriminated between nonanaphylactic and anaphylactic events, emphasizing its utility for identifying PA as a contributing factor to an acute perioperative adverse event.

The main strength of our study resides in reporting on tightly controlled time frames for collecting tryptase samples, a feature that is usually lacking from real-life studies on PA. Limitations include the retrospective design, the small cohort size, and the single-center design. A special feature was the initial search in the Immunology laboratory database to identify patients with at least two tryptase determinations, thus excluding those with no or single tryptase determination. However, this could have

introduced a bias in the selection of patients experiencing a perioperative SHR, as some of them might not have been sampled twice for tryptase determination. Therefore, prospective multicenter studies are needed to validate the current findings. We also report that mature tryptase determinations did not improve the sensitivity for detecting PA as compared with total tryptase levels in acute and baseline samples analyzed by the consensus algorithm, $sAT > ([1.2 \times sBT] + 2) \mu\text{g/L}$.

In conclusion, better use of measuring serum tryptase levels during acute (0.5–2 hours after the initiation of the symptoms) and baseline (before onset or at least 24 hours after complete resolution of the symptoms) time periods, and analyzed by the consensus algorithm, might increase the diagnostic accuracy of PA and promote referrals to an allergist for a further workup.

AUTHOR CONTRIBUTIONS

ML, JV, and LA designed the study and accomplished ethical formalities. LA, MG, LZ, JA, NB, DL, and ML performed anesthesiology and allergy evaluation. LBS and JV designed, supervised, collected, and analyzed laboratory workup. LA, SM, and JV collected and analyzed clinical data. LA, LBS, JV, and ML wrote the paper. PMM and JLM revised the paper for important intellectual content. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

JV has received speaker honoraria from MEDA Pharma—Mylan and consultancy fees from Sanofi. Virginia Commonwealth University receives royalties from Thermo Fisher for the tryptase assay and shares those with LBS, its inventor. ML has received personal fees, grants, and nonfinancial support outside this work from MSD, Pfizer, Amomed, Aguetant. The other authors declare no conflict of interest.

ORCID

Joana Vitte  <https://orcid.org/0000-0002-3344-9408>

REFERENCES

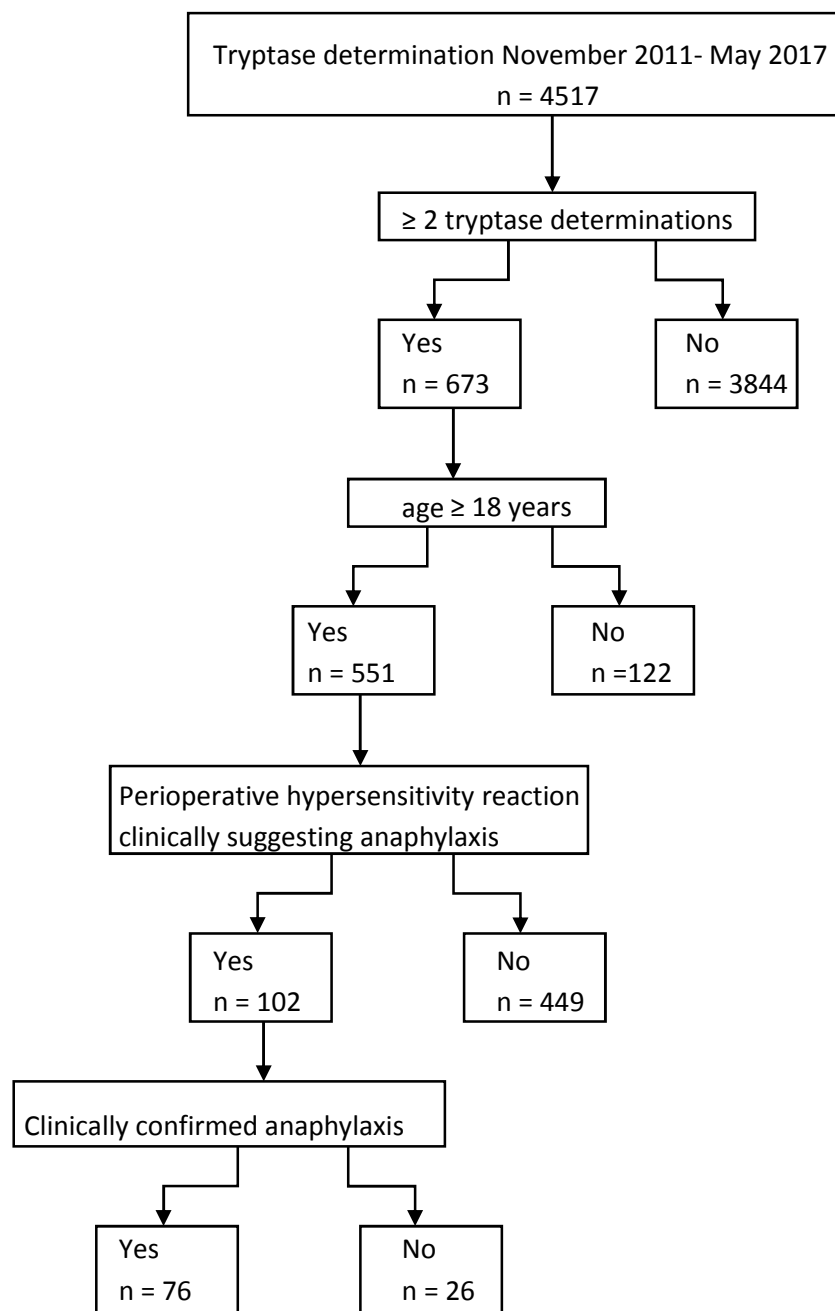
1. Lieberman P, Nicklas RA, Randolph C, et al. Anaphylaxis—a practice parameter update 2015. *Ann Allergy Asthma Immunol*. 2015;115:341–384.
2. Freundlich RE, Duggal NM, Housey M, Tremper TT, Engoren MC, Kheterpal S. Intraoperative medications associated with hemodynamically significant anaphylaxis. *J Clin Anesth*. 2016;35:415–423.
3. Saager L, Turan A, Egan C, et al. Incidence of intraoperative hypersensitivity reactions: a registry analysis. *Anesthesiology*. 2015;122:551–559.
4. Simons FER, Ebisawa M, Sanchez-Borges M, et al. 2015 update of the evidence base: World Allergy Organization anaphylaxis guidelines. *World Allergy Organ J*. 2015;8:1–16.
5. Mertes PM, Malinovsky JM, Jouffroy L, Aberer W, Terreehorst I, et al. Reducing the risk of anaphylaxis during anesthesia: 2011 updated guidelines for clinical practice. *J Invest Allergol Clin Immunol*. 2011;21:442–453.

6. Harper NJN, Cook TM, Garcez T, et al. Anaesthesia, surgery, and life-threatening allergic reactions: epidemiology and clinical features of perioperative anaphylaxis in the 6th National Audit Project (NAP6). *Br J Anaesth*. 2018;121:159-171.
7. Muraro A, Roberts G, Worm M, et al. Anaphylaxis: guidelines from the European Academy of Allergy and Clinical Immunology. *Allergy*. 2014;69:1026-1045.
8. Ebo DG, Faber M, Elst J, et al. In vitro diagnosis of immediate drug hypersensitivity during anesthesia: a review of the literature. *J Allergy Clin Immunol Pract*. 2018;6:1176-1184.
9. 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;83:1551-1555.
10. Valent P, Akin C, Arock M, 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;157:215-225.
11. Schwartz LB, Sakai K, Bradford TR, 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;96:2702-2710.
12. Schwartz LB, Min HK, Ren S, et al. Tryptase precursors are preferentially and spontaneously released, whereas mature tryptase is retained by HMC-1 cells, Mono-Mac-6 cells, and human skin-derived mast cells. *J Immunol*. 2003;170:5667-5673.
13. Borer-Reinhold M, Haeberli G, Bitzenhofer 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;41:1777-1783.
14. 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;46:1465-1473.
15. <http://www.phadia.com/en-GB/5/Products/ImmunoCAP-Assays/ImmunoCAP-Tryptase>. Accessed April 21, 2018.
16. Tacquard C, Collange O, Gomis P, et al. Anaesthetic hypersensitivity reactions in France between 2011 and 2012: the 10th GERAP epidemiologic survey. *Acta Anaesthesiol Scand*. 2017;61:290-299.
17. Baretto RL, Beck S, Heslegrave J, et al. Validation of international consensus equation for acute serum total tryptase in mast cell activation: a perioperative perspective. *Allergy*. 2017;72:2031-2034.
18. Egner W. Adherence to recommended UK sampling protocols for detecting mast cell mediator release in perioperative anaphylaxis. *J Clin Pathol*. 2017;70:85-88.
19. Simons FE, Arduzzo LR, Bilò MB, et al. International consensus on (ICON) anaphylaxis. *World Allergy Organ J*. 2014;7:9.
20. Simons FE, Arduzzo LR, Bilò MB, et al. World allergy organization guidelines for the assessment and management of anaphylaxis. *World Allergy Organ J*. 2011;4:13-37.
21. Demoly P, Adkinson NF, Brockow K, et al. International Consensus on drug allergy. *Allergy*. 2014;69:420-437.
22. Ring J, Messmer K. Incidence and severity of anaphylactoid reactions to colloid volume substitutes. *Lancet*. 1977;1:466-469.
23. Owens WD, Felts JA, Spitznagel EL Jr. ASA physical status classifications: a study of consistency of 20 ratings. *Anesthesiology*. 1978;49:239-243.
24. Hackett NJ, De Oliveira GS, Jain UK, Kim JY. ASA class is a reliable independent predictor of medical complications and mortality following surgery. *Int J Surg*. 2015;18:184-190.
25. Schwartz LB, Bradford TR, Rouse C, et al. Development of a new, more sensitive immunoassay for human tryptase: use in systemic anaphylaxis. *J Clin Immunol*. 1994;14:190-204.
26. Castells M. Diagnosis and management of anaphylaxis in precision medicine. *J Allergy Clin Immunol*. 2017;140:321-333.
27. Pastorello EA, Farioli L, Losappio LM, 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.
28. Morici N, Farioli L, Losappio LM, et al. Mast cells and acute coronary syndromes: relationship between serum tryptase, clinical outcome and severity of coronary artery disease. *Open Heart*. 2016;3:e000472.
29. Maaninka K, Nguyen SD, Mäyränpää MI, et al. Human mast cell neutral proteases generate modified LDL particles with increased proteoglycan binding. *Atherosclerosis*. 2018;275:390-399.
30. Prieto-García A, Pérez-David E, Devesa C, et al. Fatal anaphylaxis caused by gadolinium due to beta-tryptase-induced hemorrhagic diathesis. *J Allergy Clin Immunol Pract*. 2017;5:1433-1434.
31. Piliponsky AM, Romani L. The contribution of mast cells to bacterial and fungal infection immunity. *Immunol Rev*. 2018;282:188-197.
32. Campillo-Navarro M, Leyva-Paredes K, Donis-Maturano L, et al. *Mycobacterium tuberculosis* catalase inhibits the formation of mast cell extracellular traps. *Front Immunol*. 2018;9:1161.
33. Krishna MT, York M, Chin T, et al. Multi-centre retrospective analysis of anaphylaxis during general anaesthesia in the United Kingdom: aetiology and diagnostic performance of acute serum tryptase: Anaphylaxis during 19 general anaesthesia. *Clin Exp Immunol*. 2014;178:399-404.
34. Brown SG, Blackman KE, Heddl RJ. Can serum mast cell tryptase help diagnose anaphylaxis? *Emerg Med Australas*. 2004;16:120-124.
35. Vegh AB, George KC, Lotfi-Emran S, Butler NE, Schwartz LB. Total tryptase levels indicate risk for systemic reactions to rush immunotherapy and mast cell activation. *Ann Allergy Asthma Immunol*. 2011;106:342-343.
36. Laroche D, Gomis P, Gallimidi E, Malinovsky J-M, Mertes PM. Diagnostic value of histamine and tryptase concentrations in severe anaphylaxis with shock or cardiac arrest during anesthesia. *Anesthesiology*. 2014;121:272-279.
37. Lyons JJ, Yu X, Hughes JD, et al. Elevated basal serum tryptase identifies a multisystem disorder associated with increased TPSAB1 copy number. *Nat Genet*. 2016;48:1564-1569.
38. Lyons JJ, Sun G, Stone KD, et al. Mendelian inheritance of elevated serum tryptase associated with atopy and connective tissue abnormalities. *J Allergy Clin Immunol*. 2014;133:1471-1474.
39. Mirone C, Preziosi D, Mascheri A, et al. Identification of risk factors of severe hypersensitivity reactions in general anaesthesia. *Clin Mol Allergy*. 2015;13:11.
40. Egner W, Cook TM, Garcez T, 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;48:846-861.
41. Laroche D, Bricard H, Laxenaire MC. [Allergo-anesthesia consultation: not enough patients are tested after an anaphylactoid anesthetic incident]. *Ann Fr Anesth Reanim*. 1998;17:89-90.
42. Gurrieri C, Weingarten TN, Martin DP, et al. Allergic reactions during anesthesia at a large United States referral Center. *Anesth Analg*. 2011;113:1202-1212.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Vitte J, Amadei L, Gouitaa M, et al. Paired acute-baseline serum tryptase levels in perioperative anaphylaxis: An observational study. *Allergy*. 2019;74:1157-1165. <https://doi.org/10.1111/all.13752>



Supplemental Figure 1. Study flowchart.

Patients aged 18 years or more, having experienced a perioperative reaction clinically suggesting anaphylaxis from November 2011 through May 2017, and for whom at least two tryptase determinations had been performed, were retrospectively identified. Following the EAACI and ICON clinical criteria, anaphylaxis was confirmed in 76 (75%) patients.

Patient number	Age	M/F	Perioperative anaphylaxis	Acute sample		Baseline sample	1.2 x sBT + 2 algorithm in favor of MC degranulation
				total tryptase	mature tryptase	total tryptase	
1	52	F	0	7.04	<1	4.54	No
2	88	F	0	4.61	<1	4.13	No
3	66	M	0	3.66	<1	2.48	No
4	67	F	0	6.11	<1	4.24	No
5	53	M	0	8.65	<1	3.66	Yes
6	63	F	0	3.85	<1	3.55	No
7	82	F	0	8.29	<1	4.28	Yes
8	29	F	0	2.22	<1	1.24	No
9	62	M	0	2.42	<1	2.21	No
10	54	M	0	2.89	<1	2.94	No
11	37	F	1	2.46	<1	2.31	No
12	88	F	1	8.2	<1	6.13	No
13	29	F	1	2.51	<1	3.09	No
14	26	F	1	5.91	<1	3.43	No
15	72	F	2	9.43	<1	2.35	Yes
16	51	F	2	7.07	<1	1.94	Yes
17	57	M	2	8.99	<1	2.07	Yes
18	70	M	2	9.00	<1	3.39	Yes
19	35	M	2	2.64	<1	1.6	No
20	21	F	2	3.82	<1	3.14	No
21	62	F	2	2.02	<1	1.01	No
22	67	M	2	10.4	<1	3.72	Yes
23	37	F	3	10.6	<1	4.9	Yes
24	67	M	3	1.96	<1	2.12	No
25	44	F	3	5.88	<1	4.62	No
26	38	M	3	10.6	<1	5.07	Yes
27	79	M	3	9.35	<1	5.93	Yes
28	43	F	3	3.95	<1	1.22	Yes
29	32	F	3	8.49	<1	4.11	Yes
30	60	M	3	2.44	<1	2.69	No
31	72	M	3	8.47	<1	3.64	Yes
32	52	M	3	12.8	<1	2.63	Yes
33	81	F	4	4.55	<1	2.83	No
34	83	F	4	3.06	<1	3.42	No
35	86	F	4	10.2	<1	10.2	No
36	72	F	4	11.3	<1	3.19	Yes
37	36	F	3	16.3	1.1	3.16	Yes
38	34	F	3	16.3	1.3	3.95	Yes
39	58	F	3	16.5	2.9	3.30	Yes
40	55	F	3	39.0	9	9.02	Yes
41	66	F	3	93.1	20	8.04	Yes
42	62	F	4	37.8	4.1	8.04	Yes

3 **Supplemental Table 1. Mature tryptase determination.** For a study subgroup of 42 patients who
4 had experienced a perioperative hypersensitivity reaction clinically suggesting anaphylaxis and had
5 been initially evaluated for acute and baseline total tryptase (ImmunoCAP Thermo Fisher) in the
6 Marseille Laboratory, acute samples were assayed for mature tryptase at the VCU Laboratory.
7 Patients with detectable mature tryptase in the acute sample (6/42) are shown in bold font.

Supplemental Material: Allergy work-up

An allergist (MG) formally identified the culprit drug in 28 of the 38 perioperative anaphylaxis (PA) patients evaluated, failing to identify it in 10 patients. Six non anaphylactic patients referred for allergy work-up were confirmed as non anaphylactic and non allergic. In 12 (12%) patients who had experienced a perioperative systemic hypersensitivity reaction (SHR) clinically suggesting anaphylaxis and who were not referred to the allergy clinic, identification of the culprit drug was considered as reliable based on the clinical findings, the time frame of symptom initiation, a personal history of previously unreported drug allergy and the results of specific IgE. Overall, the culprit was identified in 40 (53%) patients among the 76 who met clinical criteria for PA.

Prior to allergy work-up, at least one trigger was suspected in each patient. Hypnotic drugs (77%), opioids (68%), NMBA (47%) and antibiotics (34%) were the most frequently suspected drugs, based on the nature of the perioperative drugs received by the patient and the time frame of symptom initiation after drug administration. Confirmed culprits were NMBA, antibiotics, latex, and gelatin in 18 (28%), 13 (20%), 9 (14%), and 2 (3%) patients, respectively. Antibiotics and NMBA were associated with grade 3 and 4 PA (Supplemental Table 2). An association of two culprits was found in two patients from the antibiotic group: antibiotic and NMBA, and antibiotic and latex.

The consensus algorithm confirmed perioperative MC degranulation in 17/18 NMBA cases (1 patient with inadequate and uninterpretable tryptase results), in 12/13 antibiotic cases (1 false negative result for grade 3 anaphylaxis with sAT of 3.5 µg/L and sBT of 2.1 µg/L) and in 6/9 latex cases (1 false negative result for grade 3 anaphylaxis with a sAT of 5.91 and sBT of 3.43, and 2 patients with inadequate and uninterpretable tryptase results). Confirmed NMBA anaphylaxis was associated with higher sAT levels (median 25.2 µg/L, IQR 12.1-48.9, range 2.0-339), as compared with sAT in non-NMBA anaphylaxis (median 9.0 µg/L, IQR 3.8-23.3, range 2.0-164), $p = 0.01$. sAT levels in antibiotic-induced and latex-induced anaphylaxis followed a similar trend, but did not reach the significance level.

Among NMBA, succinylcholine (9/18), cisatracurium (1/18) and rocuronium (1/18) were the most prevalent culprits. Rocuronium-triggered anaphylaxis was less prevalent than in previous studies (1;2-3). Antibiotics were a relatively frequent trigger of PA with a prevalence of 28%, confirming other studies and reflecting the evolution of antibiotic prophylaxis in surgery (1-2;4-8). In the antibiotic group, seven (54%) patients were allergic to penicillins and two (15%) to cephalosporins. Interestingly, in two patients, the culprit was identified as gelofusin, a 4% suspension of gelatin employed for the prevention or treatment of hypovolemia. Hypnotics and opioids were more often

suspected than NMBA and antibiotics, as opposed to previous data (1;8). After an allergy work-up, no hypnotic or opioid drug was recognized as a cause of PA in our cohort.

Supplemental references

1. Mertes PM, Laxenaire M-C, Alla F, Groupe d'Etudes des Réactions Anaphylactoïdes Peranesthésiques. Anaphylactic and anaphylactoid reactions occurring during anesthesia in France in 1999-2000. *Anesthesiology* 2003;99:536–45.
2. Reddy JI, Cooke PJ, van Schalkwyk JM, Hannam JA, Fitzharris P, Mitchell SJ. Anaphylaxis is more common with rocuronium and succinylcholine than with atracurium. *Anesthesiology* 2015;122:39–45.
3. Lobera T, Audicana MT, Pozo MD, Blasco A, Fernández E, Cañada P *et al.* Study of hypersensitivity reactions and anaphylaxis during anesthesia in Spain. *J Investig Allergol Clin Immunol* 2008;18:350–6.
4. 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.
5. Laxenaire MC, Mertes PM; Groupe d'Etudes des Réactions Anaphylactoïdes Peranesthésiques. Anaphylaxis during anaesthesia. Results of a two-year survey in France. *Br J Anaesth* 2001;87:549-58.
6. Dong SW, Mertes PM, Petitpain N, Hasdenteufel F, Malinovsky JM; GERAP. Hypersensitivity reactions during anesthesia. Results from the ninth French survey (2005-2007). *Minerva Anesthesiol* 2012;78:868-78.
7. Meng J, Rotiroti G, Burdett E, Lukawska JJ. Anaphylaxis during general anaesthesia: experience from a drug allergy centre in the UK. *Acta Anaesthesiol Scand* 2017;61:281–9.
8. Kemp HI, Cook TM, Thomas M, Harper NJN. UK anaesthetists' perspectives and experiences of severe perioperative anaphylaxis: NAP6 baseline survey. *Br J Anaesth* 2017;119:132–9.

	No anaphylaxis	Perioperative anaphylaxis					Multiple t test (<i>p</i>)
		Grade 1	Grade 2	Grade 3	Grade 4	All	
Positive skin tests to culprit drug	0/6	25% (1/4)	50% (4/8)	82% (18/22)	100% (4/4)	71% (27/38)	0.002
Positive specific IgE to culprit drug	0/16	38% (3/8)	33% (4/12)	61% (22/36)	50% (3/6)	52% (32/62)	0.006
Confirmed antibiotic	0/18	0/8	0/12	28% (10/36)	38% (3/8)	20% (13/64)	0.008
Confirmed NMBA	0/18	0/8	8% (1/12)	42% (15/36)	25% (2/8)	28% (18/64)	0.002
Confirmed latex	0/18	25% (2/8)	17% (2/12)	14% (5/36)	0/8	14% (9/64)	0.2

57

58 **Supplemental Table 2. Confirmed culprits of perioperative anaphylaxis.**

59 Results are shown for the subsets of patients having undergone laboratory (n=78) or clinical (n=44)
60 allergy work-up. The culprit was identified in 40 patients among 76 clinically confirmed perioperative
61 anaphylaxis.