Brief report

Extending the role of tryptase in perioperative anaphylaxis: Predicting positive results in basophil activation tests

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Background: Basophil activation tests (BATs) are useful in identifying culprits of perioperative anaphylaxis (PA), but their utility remains limited due to technical limitations, cost, and availability. Being able to prioritize patients with likely higher yields for BAT would be useful in reducing costs and manpower.
Objective: We sought to investigate whether tryptase levels and clinical parameters may be useful for selecting patients for BATs.
Methods: We performed a 10-year retrospective study in Hong Kong to investigate the performance of BATs associated with tryptase levels (taking during PA) and other clinical parameters.
Results: Of 90 patients, 70 (77.8%) showed significant tryptase level elevation and 37 (41.1%) had a positive BAT result. BAT-positive patients presented with significantly higher absolute levels (15.9 μg/L vs 9.1 μg/L; $P = .018$), absolute elevation (12.8 μg/L vs 7.1 μg/L; $P = .012$), and fold elevation (5.6- vs 4.1-fold; $P = .014$) of acute tryptase than did BAT-negative patients. Among patients with positive BAT result, 94.6% (35 of 37) demonstrated elevated acute tryptase, significantly more than the BAT-negative group (66.0%; $P < .001$). In regression analysis, tryptase elevation was the sole significant factor correlated to BAT positivity (odds ratio, 10.14; 95% CI, 2.15-47.85; $P = .003$). Overall, elevated acute tryptase demonstrated a sensitivity of 94.7% and a negative predictive value of 90.0% in predicting positive results with BATs.
Conclusions: We observed that tryptase elevation is a very sensitive predictor of BAT positivity among patients with identified culprits of PA. Acute elevation of tryptase would not only aid in confirming anaphylaxis but may also help guide the decision toward selecting labor-intensive and costly *in vitro* tests such as BATs. (J Allergy Clin Immunol Global 2024;3:100297.)

Key words: *Perioperative, anaphylaxis, hypersensitivity, tryptase, basophil activation test*

Abbreviations used
BAT: Basophil activation test
MC: Mast cell
PA: Perioperative anaphylaxis
sIgE: Specific IgE
ST: Skin test

INTRODUCTION

During the perioperative period, various complications, which may interrupt critical operations and be life-threatening, may occur. Perioperative anaphylaxis (PA) is an uncommon, but potentially lethal, immediate-type hypersensitivity reaction with an associated mortality of around 5%.[1] PA may be frequently "missed" or misdiagnosed with various non–immune-mediated reactions. Evidence of an elevated tryptase is specific for mast cell (MC) degranulation and can be of immense diagnostic value.[2] Unfortunately, measuring this time-sensitive tryptase sample acutely during PA is often forgotten (or not requested) and may be "falsely negative" in up to 25% of episodes.[3]

Following the diagnosis of PA, identification of specific culprits has traditionally relied on *in vivo* testing such as skin tests (STs) and/or drug provocation tests. Given the limitations of STs and difficulties of drug provocation tests with anesthetic agents, *in vitro* tests such as specific IgE (sIgE) and basophil activation tests (BATs) have grown in popularity.[2] Although BATs are generally considered specific, they suffer from low sensitivity and are less useful in ruling out potential culprits.[2] Furthermore, BATs require fresh samples and laboratory expertise and are not readily available beyond research institutes. Therefore, being able to prioritize patients with likely higher yields for BATs would be useful in reducing unnecessary costs and manpower. We previously observed that the performance of BATs may be poorer among patients with nonelevated tryptase levels, which may be a useful parameter for selecting patients for BATs.[4] Therefore, we performed this 10-year retrospective study to further explore this phenomenon.

This study was a retrospective chart review of all adult patients with confirmed PA who completed workup at the General Anaesthesia Clinic of Queen Mary Hospital between January 2012 and December 2021. Queen Mary Hospital is a tertiary referral center accepting both public and private referrals from

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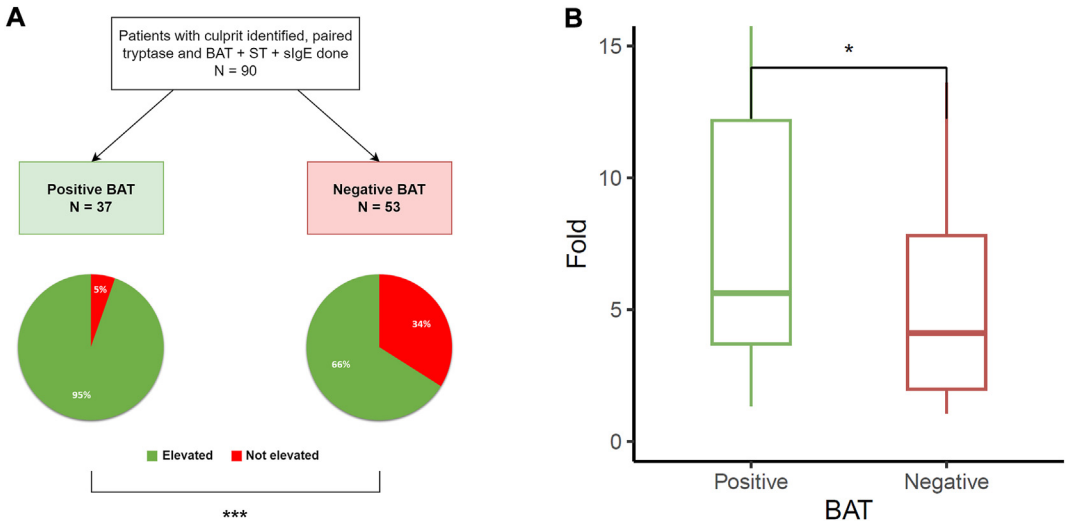

FIG 1. **A**, Acute tryptase elevation according to BAT results. **B**, Fold changes in tryptase (between baseline and acute levels) according to BAT results. *$P < .05$; **$P < .01$; ***$P < .001$.

TABLE I. Tryptase parameters stratified by BAT results

Patients with culprit identified	Total (N = 90)	Positive BAT result (N = 37)	Negative BAT result (N = 53)	*P* value
Significant tryptase rise	70 (77.8)	35 (94.6)	35 (66.0)	.001*
Acute tryptase level (μg/L)	12.5 (7.3-25.9)	15.9 (10.1-29.3)	9.1 (3.7-24.4)	.018*
Baseline tryptase level (μg/L)	2.9 (1.7-4.0)	3.0 (1.7-4.3)	2.7 (1.7-3.8)	.409
Acute/baseline (fold) tryptase, times	4.9 (2.9-10.0)	5.6 (3.6-12.2)	4.1 (1.7-8.0)	.014*
Δ tryptase (acute – baseline) (μg/L)	9.8 (4.4-23.8)	12.8 (8.0-25.0)	7.1 (1.7-21.4)	.012*

Numbers were expressed in median (lower quartile – upper quartile) or number (percentage) as appropriate.
*Stastical significance.

across Hong Kong. It is also the only center in the territory offering formal allergy and immunology testing service for suspected PA. Our diagnostic approach and technical details have been previously reported, with STs, sIgE, and BATs routinely performed for all patients.[5] We included only those patients who had paired tryptase samples available (acute [<4 hours following PA] and baseline [>24 hours]), completed STs, sIgE, and BATs with all possible culprits, and were diagnosed with a specific culprit (allergist-diagnosed with compatible history and concordant allergy investigations). Patients with PA but where an exact culprit could not be identified with more than 1 diagnostic modality (ST, sIgE, BAT, or provocation testing) and as per allergist diagnosis were excluded. Patients who did not complete full workup or have complete investigation results were also excluded. Significant tryptase elevation was defined as acute level greater than or equal to 1.2 × baseline level + 2 μg/L.[6] In those patients without a significant tryptase rise, PA was clinically diagnosed as per documented objective parameters (vital signs, examination findings) documented in the operative records and met diagnostic criteria for anaphylaxis. Patient demographics, clinical details of the index reaction, and results of all investigations were analyzed. Most statistical analyses were done using IBM SPSS Statistics version 28.0 (IBM Co, Armonk, NY). Association analysis was performed with chi-square test for categorical variables and Mann-Whitney *U* test for continuous variables, respectively, followed by multivariate regression analysis. A receiver-operating characteristic analysis was performed to evaluate the performance of acute tryptase level and significant tryptase elevation in predicting BAT positivity via the *ROCit* package on R version 4.3.1 (R Foundation, Vienna, Austria).[7] Based on their area under the curve, their prediction performance was classified into "excellent" (0.900-1.000), "very good" (0.800-0.899), "good" (0.700-0.799), satisfactory (0.600-0.699), or "unsatisfactory" (0.500-0.599).[8] An optimal cutoff point was defined as the acute tryptase level with the highest Youden's index (sensitivity + specificity).[8] *P* value less than .05 is considered statistically significant. This study was approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster.

RESULTS AND DISCUSSION

A total of 90 patients with an exact culprit identified and meeting all inclusion criteria were included for analysis. Seventy (77.8%) showed significant tryptase elevation (Fig 1, *A*). In 41.1% (37 of 90) of patients, BAT results were positive to the identified culprit. BAT-positive patients presented with significantly higher absolute levels (15.9 μg/L vs 9.1 μg/L; $P = .018$), absolute elevation (12.8 μg/L vs 7.1 μg/L; $P = .012$), and fold elevation (5.6- vs 4.1-fold; $P = .014$) of acute tryptase than BAT-negative patients (Fig 1, *B*; Table I). Among patients with a positive BAT result, 94.6% (35 of 37) demonstrated elevated acute tryptase, significantly more than the BAT-negative group (66.0%; $P < .001$). Aside from acute tryptase results, no significant differences were noted between BAT-positive and BAT-negative patients (Table II). In regression analysis, tryptase elevation was the sole

TABLE II. Demographic and clinical characteristics of analyzed patients with PA

Patients with culprit identified	Total (N = 90)	Positive BAT result (N = 37)	Negative BAT result (N = 53)	*P* value
Demographic characteristics				
Male sex	44 (48.9)	18 (48.6)	26 (49.1)	.970
Age of onset (y)	58.8 (48.5-65.9)	60.0 (50.9-69.8)	57.3 (44.6-64.3)	.113
Han Chinese ethnicity	89 (98.9)	36 (97.3)	53 (100.0)	.411
Interval between reaction and workup	1.7 mo (1.3-3.1)	1.6 mo (1.2-3.1)	2.0 mo (1.4-3.2)	.080
Past medical history				
Previous use of GA	33 (36.7)	14 (37.8)	19 (35.8)	.847
Hypertension	33 (36.7)	12 (32.4)	21 (39.6)	.486
Asthma ± COPD	10 (11.1)	3 (8.1)	7 (13.2)	.516
Autoimmune disease	5 (5.6)	2 (5.4)	3 (5.7)	1.000
Chronic urticaria	9 (10.0)	4 (10.8)	5 (9.4)	1.000
Previous drug allergy	17 (18.9)	6 (16.2)	11 (20.8)	.588
ASA grading				.896
Grade 1	18 (22.0)	9 (25.7)	9 (19.1)	
Grade 2	39 (47.6)	16 (45.7)	23 (48.9)	
Grade 3	23 (28.0)	9 (25.7)	14 (29.8)	
Grade 4	2 (2.4)	1 (2.9)	1 (2.1)	
Timing				.136
Induction	51 (56.7)	18 (48.6)	33 (62.3)	
Maintenance	31 (34.4)	17 (45.9)	14 (26.4)	
Recovery	8 (8.9)	2 (5.4)	6 (11.3)	
Clinical manifestations				
Respiratory	40 (44.4)	16 (43.2)	24 (45.3)	.848
Cardiovascular	80 (88.9)	35 (94.6)	45 (84.9)	.188
Mucocutaneous	51 (56.7)	19 (51.4)	32 (60.4)	.395
Ring & Messmer anaphylaxis grading				
Grade 1	4 (4.4)	0 (0.0)	4 (7.5)	
Grade 2	2 (2.2)	1 (2.7)	1 (1.9)	
Grade 3	79 (87.8)	32 (86.5)	47 (88.7)	
Grade 4	5 (5.6)	4 (10.8)	1 (1.9)	
(severe, ie, grade 3/4)	84 (93.3)	36 (97.3)	48 (90.6)	.394
Operation completed	60 (66.7)	24 (64.9)	36 (67.9)	.762
Other allergy tests				
Positive ST result	80 (88.9)	31 (83.8)	49 (92.5)	.307
Positive sIgE	27 (30.3)	15 (40.5)	12 (22.6)	.068

Numbers are expressed in median (lower quartile – upper quartile) or number (percentage) as appropriate.
ASA, American Society of Anesthesiologists; *COPD*, chronic obstructive pulmonary disease; *GA*, general anesthesia.

significant factor correlated to BAT positivity (odds ratio, 10.14; 95% CI, 2.15-47.85; $P = .003$). In receiver-operating characteristic analysis, both acute tryptase level (area under the curve, 0.647; 95% CI, 0.529-0.765) and significant tryptase elevation (area under the curve, 0.646; 95% CI, 0.528-0.764) demonstrated satisfactory performance for BAT positivity prediction (Fig 2). An acute tryptase level of greater than or equal to 9.0 μg/L was identified as the optimal cutoff, which has a sensitivity of 89.2%, a specificity of 50.0%, and a negative predictive value of 86.7% (see Table E1 in this article's Online Repository at www.jaci-global.org). Overall, elevated acute tryptase demonstrated a sensitivity of 94.7%, a specificity of 34.6%, and a negative predictive value of 90.0% in predicting positive results with BATs. In contrast, results of STs or sIgE were not significantly associated with acute tryptase results (neither numerically, absolute-change, nor fold-change; Table III).

To our knowledge, this is the first study to investigate the associations between tryptase elevation and the performance of BATs among patients with PA. In our large cohort of patients with PA undergoing BATs, almost all (94.6%) positive results came from patients with significantly elevated tryptase during the index reaction. This phenomenon is reminiscent of previous reports describing the association between BAT positivity and elevated

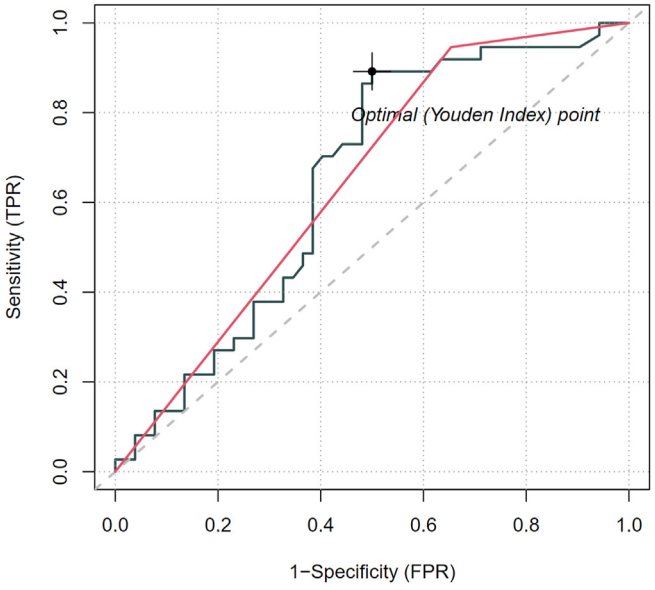

FIG 2. Performance of acute tryptase level and significant tryptase elevation in predicting positive BAT results. *FPR*, False-positive rate; *TPR*, true-positive rate.

TABLE III. Tryptase parameters stratified by ST or sIgE results

Patients with culprit identified	Total	Positive test result	Negative test result	*P* value
ST	N = 90	N = 80	N = 10	
Significant tryptase rise	70 (77.8)	64 (80.0)	6 (60.0)	.220
Acute tryptase level (μg/L)	12.5 (7.3-25.9)	14.4 (7.5-26.2)	10.6 (3.6-23.1)	.367
Baseline tryptase level (μg/L)	2.9 (1.7-4.0)	2.9 (1.7-4.1)	2.4 (1.3-3.6)	.394
Acute/baseline (fold) tryptase, times	4.9 (2.9-10.0)	4.9 (2.9-9.5)	4.7 (1.2-12.9)	.668
Δ tryptase (acute – baseline) (μg/L)	9.8 (4.4-23.8)	10.3 (4.7-23.8)	8.0 (0.7-21.4)	.311
sIgE	N = 90	N = 27	N = 63	
Significant tryptase rise	70 (77.8)	22 (81.5)	48 (76.2)	.580
Acute tryptase level (μg/L)	12.5 (7.3-25.9)	14.7 (8.2-27.4)	11.6 (5.9-25.7)	.672
Baseline tryptase level (μg/L)	2.9 (1.7-4.0)	3.3 (1.7-5.8)	2.8 (1.7-3.7)	.369
Acute/baseline (fold) tryptase, times	4.9 (2.9-10.0)	4.4 (2.5-11.2)	5.1 (2.9-9.8)	.954
Δ tryptase (acute – baseline) (μg/L)	9.8 (4.4-23.8)	10.4 (5.8-24.1)	9.6 (3.4-23.7)	.745

Numbers are expressed in median (lower quartile – upper quartile) or number (percentage) as appropriate.

tryptase among patients experiencing reactions during desensitization with platinum compounds.[9] Because tryptase exists (almost exclusively) within both MCs and basophils, significant elevation during PA may reflect the individual tendency for MC/basophil degranulation (in context with the specific drug culprit).[10] Therefore, such individuals would have an increased likelihood of positive BAT result with the same drug, in comparison to those without significantly elevated tryptase levels. Furthermore, recent reports suggest a close interplay between MCs and basophils, and it is possible that a positive BAT result reflects a more significant role of basophils in activating MCs during PA in certain susceptible individuals.[11] Interestingly, this may partly explain why the same associations with elevated tryptase levels were not observed with positive ST result or sIgE. It would be of great interest to investigate whether similar phenomena can be observed in MC activation test assays or within the context of non–drug-induced anaphylactic reactions.

There are several limitations to this study. The performance of BAT is dependent on multiple other factors, including individual laboratory variations, basophil counts, patient comorbidities, and specific culprits, which were not studied. It should be noted that BAT has varying sensitivity and specificity for different causes of PA, with fairly satisfactory performance against, for instance, neuromuscular-blocking agents and chlorhexidine, but not β-lactam antibiotics.[5,12-14] We did not specifically look into potential differences in CD63 and CD203c expression. The exact timing of tryptase sampling (within the 4-hour window) was not available either. Furthermore, not all identified culprits were confirmed by drug provocation tests. Despite the fact that all included patients had an exact culprit identified by multiple diagnostic modalities, which were concordant with a clinical diagnosis made by an allergist, there remains a possibility of false-positive results. Prospective and multicenter studies will be required to overcome these limitations and evaluate this novel utility of tryptase results. Regardless, we believe that our findings are of empirical value and could help inform clinicians planning their investigations in real-life practice.

In conclusion, we observed that tryptase elevation is a very sensitive predictor of BAT positivity among patients with identified culprits of PA. Given the additional utility of tryptase levels in predicting BAT positivity, we strongly reinforce the importance of measuring acute tryptase in all instances of suspected PA. Acute elevation of tryptase would not only aid in confirming anaphylaxis but may also help guide the decision toward selecting labor-intensive and costly *in vitro* tests such as BAT.

DISCLOSURE STATEMENT

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

REFERENCES

1. Gonzalez-Estrada A, Campbell RL, Carrillo-Martin I, Renew JR, Rank MA, Volcheck GW. Incidence and risk factors for near-fatal and fatal outcomes after perioperative and periprocedural anaphylaxis in the USA, 2005-2014. Br J Anaesth 2021;127:890-6.
2. Takazawa T, Sabato V, Ebo DG. In vitro diagnostic tests for perioperative hypersensitivity, a narrative review: potential, limitations, and perspectives. Br J Anaesth 2019;123:e117-25.
3. 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.
4. Au EYL, Lau CS, Lam K, Chan E. Perioperative anaphylaxis and investigations: a local study in Hong Kong. Singapore Med J 2020;61:200-5.
5. Au EYL, Mak HWF, Yeung MHY, Chiang V, Lam K, Wong JCY, et al. Ten-year outcomes of Perioperative Anaphylaxis Workup Study in Hong Kong (PAWS-HK): performance of diagnostic modalities. Ann Allergy Asthma Immunol 2023;130:752-9.e1.
6. 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.
7. Khan MRA, Brandenburger T. ROCit: performance assessment of binary classifier with visualization. R package version 2020;2:1-21.
8. Metz CE. Basic principles of ROC analysis. Semin Nucl Med 1978;8:283-98.
9. Giavina-Bianchi P, Galvao VR, Picard M, Caiado J, Castells MC. Basophil activation test is a relevant biomarker of the outcome of rapid desensitization in platinum compounds—allergy. J Allergy Clin Immunol Pract 2017;5:728-36.
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. Korosec P, Turner PJ, Silar M, Kopac P, Kosnik M, Gibbs BF, et al. Basophils, high-affinity IgE receptors, and CCL2 in human anaphylaxis. J Allergy Clin Immunol 2017;140:750-8.e15.
12. Bennett MR, Mathioudakis AG, Wu J, Chu MMH, Tontini C, Thomas I, et al. Performance characteristics of basophil activation tests for diagnosing penicillin allergy: a meta-analysis. J Allergy Clin Immunol Pract 2024;12:714-23.e5.
13. Dewachter P, Chollet-Martin S, Mouton-Faivre C, de Chaisemartin L, Nicaise-Roland P. Comparison of basophil activation test and skin testing performances in NMBA allergy. J Allergy Clin Immunol Pract 2018;6:1681-9.
14. Tacquard C, Iba T, Levy JH. Perioperative anaphylaxis. Anesthesiology 2023;138:100-10.