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A 17 year experience in Perioperative Anaphylaxis 1998-2015: Harmonising optimal detection of Mast Cell mediator release.

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Key words: Allergy, Human, Mast Cells, Anaesthesia, Perioperative, Anaphylaxis

Abbreviations

NICE National Institute of Clinical Excellence

AAGBI Association of Anaesthetists of Great Britain and Ireland

CV coefficient of variation

CI Confidence interval

T1, T2, T3 and Tp First, second, third and peak tryptase samples

UMH Urinary Methylhistamine

WAO World Allergy Organisation

IQR interquartile range

ΔTp20% positive change in tryptase >= 20% from trough to peak

ΔTp3 positive change in tryptase level from trough to peak of >=3 μg/L

Abstract

Background

Sheffield NARCOS (National Adverse Reactions Advisory Service) investigates suspected perioperative anaesthetic reactions using serial tryptase, urinary methylhistamine and clinical information. Further recommendations for additional allergy clinic assessment are provided.

Objective

To establish a robustly measurable protocol for identifying mast cell mediator (MMR) release in this cohort. To compare these thresholds with previous suggested thresholds and algorithms.

Method

A review of 3,455 NARCOS cases referred with a suspected peri-operative allergic reaction. Tryptase, Urinary methylhistamine (UMH) and clinical details were analysed. 1746 cases were graded using the Ring and Messmer scale. Reaction grade, tryptase and UMH changes were compared with statistical and graphical presentations appropriate to non-normally distributed measurements using Analyse-IT software.

Results

Sensitive strategies such as 3μg/l or 20% are measurable, translatable and would substantially increase detection of potentially relevant changes in tryptases. Adequate quality assurance for low level measurement is needed.

An incremental threshold of 20% would identify potential MMR in an additional 14% of cases with peak tryptase (Tp) between 5 and 14 μg/L and a further 15% with Tp below 5 μg/L. Further work is required to establish the diagnostic performance characteristics of this more sensitive approach.

UMH also identified up to 120 further cases of potential MMR in absence of tryptase increments.

Conclusion and Clinical Relevance

Future studies should establish and compare the predictive performance characteristics of each strategy against clinical phenotypes. A single agreed definition of positive serial tryptases is needed to enable robust evaluation of diagnostic strategies. This could serve as a harmonised standard for comparative studies of case series from different centres.

Introduction:

The optimum number of tryptase measurements and the best interpretative strategy have not yet been established in peri-operative anaphylaxis. The predominant diagnostic indicator in allergy diagnosis is the clinical history and most tests are thought to be less reliable. However the tests, and their interpretation, are the most amenable to standardization or harmonization. There have been few large reviews of investigation of perioperative anaphylaxis and the optimal diagnostic test combination remains unknown. The reference diagnosis in those studies is predominantly based on clinical criteria derived from expert opinion and observational studies (WAO International Consensus 201 [1], National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network [2] and AAGBI Guidelines 2009 [3]. However neither clinical criteria, nor a single robust standardised tryptase interpretation protocol have been subjected to extensive clinical evaluation, and it is very difficult to remove mimics of anaphylaxis from the studies. Allergen triggers are only identified in half of the cases in many series [4] [5] [6] [7] [8] [9] [10]. We do not know if current presumptions of poor sensitivity of tryptase testing are biased by lack of specificity of the clinical criteria or insensitivity of the tests interpretive criteria.

This causes a particular problem when deriving performance characteristics for diagnostic tests in anaesthetic allergy, since a diagnostic drug challenge is often impractical. There are many mimics of the clinical picture of anaphylaxis, including direct mast cell mediator release and other causes of hypotension and bronchospasm, including the pharmacological action of the drugs themselves. Harmonisation of diagnostic test performance measurement is possible and can serve as a fixed point of reference to enable derivation of translatable performance characteristics. In addition the optimum number of tryptase samples has not been validated, although recently various sampling criteria have been proposed based on clinical opinion and knowledge of tryptase half-life data [11] [12] [13] [14] [15].

We examined the last 3,455 cases in NARCOS, a national service for the laboratory investigation of adverse anaesthetic drug reactions based in Sheffield, England, to evaluate

the performance of serial tryptase and urinary methyl histamine measurement. We compared the results against published criteria for clinical grading of reactions.

Methods

Data

We conducted a retrospective survey of the NARCOS database containing the laboratory and clinical information on all samples referred to Sheffield for NARCOS assessment, including tryptase (T) blood tests or urinary methyl histamine (UMH) tests taken at the time of reaction and 24 hours later. Our required proforma protocol advised serial tryptase measurements immediately, 1-6 hours after reaction and at 24 hours. Most patients had at least 2 serial tryptases (3400), but there was likely to be a wide variation in actual timing, with potential inaccuracies introduced in some during sample labelling, laboratory sample handing and referral onwards. Samples were therefore analysed according to their labelled temporal sequence as T1, T2 or T3.

Analysis

All statistical and graphical analysis was performed using Analyse-IT software V4.0 (Analyse-IT software ltd, Leeds, UK). Pearson's, Spearman's and Kendal's Tau were used for analysis of correlation of Tryptase with grade; Wilcoxon Median Differences for differences in the medians. Median and 95% confidence intervals are plotted because the data is not normally distributed. Analyte measurements, incremental changes and distributions were compared with reaction grade and the number of patients in each cohort who exceeded each threshold incremental changes from peak to trough were tabulated as detailed below. Performance of the threshold incremental strategies in those patients with peak tryptase above and below the "reference range" for both 11.4 (95^{th} centile) and 14μg/L (99^{th} centile) cut-off levels were compared.

Quality Control

Local tryptase assay validation data (ImmunoCAP, Sweden Diagnostics) demonstrated a coefficient of variation (CV) of 10% or less across the measurable range to 3.5μg/L (CV=5%). Therefore a change of tryptase value >=20% can be determined to be the 95% confidence interval for a real and measurable change in this assay; this was the minimum value used to determine a true change in tryptase between samples. For values of less than 3μg/L a very rigorous threshold of a 100% increase was used (a 2-fold change) for comparison, to avoid over-interpretation of changes at low levels. The 3μg/L threshold has a very similar effect to the (1.2xbaseline plus 2μg/L) threshold used in some studies. The upper limit of normal of 14μg/ml was validated locally as our 99th centile reference range (consistent with the manufacturer's data which quotes the 95th centile as 11.4μg/L). Utilising a simple incremental threshold of 3μg/L conveniently represents a 300% increase at 1 μg/L, a 100% increase at 3μg/L, a 60% increment at 5 μg/L, a 26% increment at 11.4 and a 21% increment at 14 μg/L. It also has the benefit of simplicity.

Throughout the study a single tryptase method (UniCAP, Thermo Scientific, Uppsala, Sweden) and single UMH assay (Pharmacia, Uppsala, Sweden) was used. Tryptase assays performed well in Internal quality control and external quality assurance (UK NEQAS Tryptase) and the between laboratory CV for all available analyser groups in the scheme are comparable and under 10% worldwide.

The urinary methyl histamine assay was validated in house and has a CV of <20% with a locally verified reference range cut-off.

Creatinine-corrected values were reported and positive results defined as greater than 25 μg /mmol creatinine (the 95th centile of normal urines locally validated). Creatinine assays were subjected to satisfactory UK NEQAS assessment throughout, but no EQA currently exists for UMH.

The confounding variable of sample mislabeling in sequence or timing, or variability in individual tryptase kinetics is compensated for by utilising peak to trough changes (ΔTp).

Results

Case mix and sampling

3455 cases were referred between January 1998 and May 2015 (1271 males and 2184 females). Twice as many females were referred. 12 cases were excluded because the laboratory failed to send the accompanying samples. 43 cases had single tryptase measurement and were excluded; of which 33% were >=14μg/L and 40% >=11.4μg/L.

Only 7 / 3455 (0.2 %) of the procedures were reported to have been abandoned. 41/ 3455 (1.2 %) deaths were noted (many post-operative), but definitive outcomes are not yet known for the whole cohort.

Tryptase Sampling and levels

3400 cases had 2 or more tryptase samples (98%); 71 % (2410) had a 3 tryptase series. 29% (990) had only two tryptases performed.

The first tryptase was raised in most cases, but the median peak values are relatively low, with only 232 being >100 and 88/232 > 200μg/L (2%).

1395/3400 (41% of the referred samples) had had a peak tryptase (Tp) >=14μg/L and 45.6% were >11.4μg/L on any one of the three samples (Table 1 & 2). The first raised tryptase was usually the first sample (T1): 1312 had a raised T1; 92 had a normal T1 but raised second sample (T2) and 8 (0.5%) had a normal T1 and T2 but a raised T3. Tryptase results are not normally distributed in any reaction class. Median peak (highest) tryptase in the positive cohort was 39.5μg/L (IQR 22.6-39.5). Median tryptase in all raised T1 samples was 35.5μg/L. Ratios of trough to peak tryptase ranged from 0 to 8400% increase.

Increments fail to identify the 21 (0.6%) cases where tryptase is >200μg/L (above the measuring range of the assay) throughout T1, T2 and T3 samples (i.e. not exceeding a 3μg/L or 20% change), and those where the tryptase drops less than 20% in the series. (Table 1-3). 20% changes were less sensitive than 3μg/L in raised peak tryptases. Initial

single tryptase measurements can be misleading, as a few are initially very low (<5) and give no clues to the subsequent rise.

Reaction Severity

2053 (60%) of referrals provided clinical information: 1746 referrals had sufficient clinical information to grade the reaction according to the Ring and Messmer scale a further 307 specified anaphylaxis, but without sufficient details to confidently grade.

230 (13%) of 1746 requests were grade 1 reactions: 259 (15%) were grade 2; 33% (575) were grade 3; 37% (641) were grade 4 reactions, of whom 217 (12%) presented solely with hypotension. 41 fatal Grade 5 cases (2.4%) were declared (Figure 1).

A single known mastocytosis case was identified at referral, with a clear additional acute change in tryptase levels.

In the absence of any change in referral patterns we can therefore assume that >70% of the entire cohort of referrals would be grade 3-5 reactions. By definition all those cases grading at 3 and above on the ring and Messmer scale will have sufficient features to suggest likely anaphylaxis on the NAIA/FAAN definitions [2].

Tryptase in different reaction grades

Median Tryptases and incremental tryptase levels rose with grade to grade 4 (Figure 1), but there was a wide range of peak tryptases with all grades, as previously documented. There was limited correlation between Tp and severity overall (Figure 1). The same was true of the UMH values (Figure 2). Significant differences between the median peak tryptase elevations ($p<0.0001$) between grade 1 all other reaction grades (2-5), with the exception of grade 2 to 3 ($p= 0.21$) 3 to 4 ($p= 0.62$) and 4 to 5 ($p=0.76$).

The 20% increment was most sensitive overall for detecting potential MMR, but interestingly was less sensitive in those with raised peak tryptases for either 95^{th} or 99^{th} centile reference ranges. 3μg increments were less sensitive in all combinations (Tables 1-3). Few changes in low level Tp below 5μg/L were above 100% although 20% changes were common (Tables

3a and 3b). Significant differences between the median peak to trough tryptase changes ($p<0.0001$) between grade 1 all other reaction grades (2-5), with the exception of grade 2 to 3 ($p= 0.21$) 3 to 4 ($p= 0.62$) and 4 to 5 ($p=0.76$). Thus both peak tryptase and tryptase changes behave similarly.

UMH in different reaction grades

Urinary Methyl histamine was positive in 45-68% of patients and in most patients with positive tryptases. It is more frequently positive in higher grade reactions (Figure 2 and Table 4) but overall correlation was limited.

UMH independently identifies patients with possible mediator release when there was no evidence of tryptase release, even on the most sensitive 20% criterion.

593 cases (25% of the cohort with multiple tryptases) had UMH measurements. 414 were positive for UMH (mostly UMH1). 213 UMH measurements were available for cases where the reaction was graded (Table 4 and Figure 2)

There were no strong correlation between UMH1 and T1 levels overall or in any subgroup (Correlation r all < 0.3) unlike that reported for plasma histamine. The majority of cases had simultaneous evidence of UMH and Tryptase release consistent with previous reports.

UMH is raised without detectable tryptase in 3% of cases.

100-120 cases (3% of the total cohort) were identified where UMH was raised without any detectable incremental change in tryptase (102 for 3μg/L incremental threshold, 100 for a 20% increment in tryptase and 120 for a 5μg/L threshold).

Discussion

Harmonisation of Tryptase interpretation is needed

Establishing a diagnosis of drug allergy is still an imprecise activity. There is currently no evidence based consensus on criteria for establishing the presence of mast cell mediator release (MMR). Clinical assessment has primacy, but in perioperative anaphylaxis there are many causes of hypotension and mimics of anaphylaxis, thus simply matching the WAO phenotype of anaphylaxis is of uncertain predictive value. Elevated tryptase is reported in 30-60% of reported series of anaphylaxis, but this may not translate to perioperative drug allergy or intravenous exposure. Guidelines exist to harmonise the evaluation of reactions (AAGBI, BSACI, NICE) [1] [16] [3] [17] [2] [18]. More evidence is needed for the performance characteristics of the available assays (tryptase and urinary methyl histamine) in large cohorts. Recent UK data suggests compliance with sampling recommendations can be poor [19] [20].

Tryptase presents a unique opportunity to harmonise diagnostic interpretation. There is currently a single assay in routine use worldwide currently (although there have been several methods in the literature) [9] [21] [6]. Therefore de-facto standardisation of measurement is in place.

What is the smallest incremental change that can be reliably measured?

Rises as small as 20% or 2μg/L or ROC thresholds of 5-7μg/L have been observed to correlate with anaphylactic reactions in various studies [22, 23]. A compound index (1.2xbaseline + 2μg/L) has also been proposed [13] [14].

No one can reliably measure a change less than twice the routine coefficient of variation (CV) of the assay. Nor can anyone usefully translate a change of less than 2xCV this into clinical practice worldwide. Previous studies deriving ROC curves for Tryptase in small cohorts may not be translatable to other centres [15], however incremental changes may be

more translatable if validated on large series. Furthermore, clinical anaphylaxis is reported in the “absence” of mast cell mediator release.

Incremental changes [24] [23] [22] [25] [26] between 20% and >100% have been proposed to add sensitivity. Non-anaphylactic reactions show a mean 29% change from baseline, in contrast to185% in emergency room anaphylaxis [27], allergic urticaria (49.5%) and non-steroidal drug reactions (38.2%) [28]. A 35% increment correlated well with VIT reactions [25] and a change of 2μg/L for anaphylaxis is sufficient in other scenarios [23] [22].
It is important that natural or incidental variations in tryptase baseline levels will not confound any strategy. There is very little evidence for circadian changes and the variation observed in published datasets is small. Mean changes of less than 20% have been observed in venom immunotherapy (VIT) [29], post-operative orthopaedic procedures [30], emergency room admissions and controls [31] [32]. While statistically significant, many variations are within the 2xCV limits of the total tryptase assay (20%) [32] [30]. Liver disease and alcohol consumption do not raise tryptase [33] but renal impairment [34] [35] [36], obesity [13], interference form heterophile antibodies [21] and chronic elevations of uncertain significance may (Egner personal observation).

Correlation between severity and peak tryptase or incremental changes is limited

No strong correlation was seen between peak tryptase and reaction grade overall (Pearson’s. Spearman’s r= 0.3 (CI 0.25-0.34) and Kendall’s Tau (r =0.23 (CI 0.2-0.26). The correlation between reaction severity and tryptase is always limited similar to this cohort [37] [20] [14], implying that tryptase is insensitive or there are non-allergic mimics of all grades of reaction which confound the analysis of all studies. Peak tryptases in all grades of reaction can be small and within the reference range [26]. We show a statistically significant difference in median peak tryptase (or tryptase increment) and grade 1 for all reaction grades, but not between grade 2 and 3, 3 and 4 or grade 1 and 2, possibly due to difficulties in differentiating these grades clinically.

How should Tryptase be used in establishing MMR?

The clinical criteria for anaphylaxis define features that suggest anaphylaxis clinically but do not exclude mimics. To assist in establishing mast cell mediator release in perioperative allergy, we must define a standardized and robust (i.e. translatable) approach to defining changes in markers, since derivation of ROC thresholds in previous cohorts erroneously pre-supposes definitive diagnosis of drug allergy against a validated reference standard.

The role of tryptase measurement should be to reliably identify those patients with tryptase changes that **could** represent mast cell mediator release. This large case series suggests that small changes in tryptase are robustly measurable and potentially useful. We establish that a threshold of 20% increment should be the minimum change that can be measured with certainty and that a simple increment of 3 μg/L from peak to trough may be a more specific alterative and functions similarly to the (1.2xbaseline+2) algorithm within the reference range. Both require large scale validation since they may identify potential tryptase release in 30-60% of patients with peak tryptases within the reference range. We show that a few additional cases of likely MMR (3%) can only be detected by UMH analysis. This may be a useful adjunct for lesser degrees of reaction which are less likely to produce large tryptase increments within the reference range.

No test is without false positives or false negatives; the 3μg/L strategy minimizes likely false positivity at lower levels in a similar way to previously suggested algorithms such as (1.2 x baseline) + 2μg/L, but the possibility that a 20% rise may of significance at all levels of tryptase remains to be proven. Simple peak tryptase thresholds including T>=14 μg/L, >=11.4 μg/L or lower are insensitive, with anaphylaxis and IgE mediated drug reactions seen with peak tryptases much lower than 14μg/L [26] [23] [24]. This series confirms that the half-life of tryptase appears to be markedly prolonged in some cases [28]. Despite these caveats, the evaluation of small serial changes is both rational and justified.

A peak tryptase of >=14μg/l is the "correct" threshold for identifying individuals with raised tryptase. 11.4μg/l is too sensitive since 2.5% of "normal" people will always exceed this leading to erroneous suspicion of elevation in normal people (Tryptase Kit insert http://www.phadia.com/en-GB/5/Products/ImmunoCAP-Assays/ImmunoCAP-Tryptase/ and Sheffield local validation data). Utilising incremental changes largely negates this disadvantage and gives similar performance irrespective of the threshold used (Tables 1-3).

Both strategies are independent of assumptions about accuracy of sample labelling timing or sequence. They make no assumptions about the kinetics of clearance. Laboratories wishing to interpret changes to the 3μg/L threshold will need to establish extra IQC strategies for very low values that are not in place routinely.

Increments are less useful in raised tryptases

Utilising increments in tryptase will only miss those cases with slow clearance or inappropriately short intervals between samples. Acute rises always fall, thus tryptase changes of greater than 3μg/l can be expected in almost all of these raised samples (except 21 case where tryptase remained >200μg/l throughout (i.e. above the upper measuring limit of the assay). 20% increments (or the 1.2xBaseline+2 algorithm) fare slightly less well because the absolute size of a 20% increment at 100μg/l is 20μg/l and at 50 it is 10μg/l.

The role of Urinary Methyl Histamine

We show that raised UMH can be seen in the absence of significantly raised tryptase [38] [13]. Further studies are needed to determine if UMH might identify additional cases of mast cell mediator release [39] [38]. In our cohort, 80% of patients with tryptase release had simultaneous UMH release. Conversely 3% had no evidence of tryptase increments (on any measure) but had positive UMH levels. This additional information may be useful increment in diagnosis and risk assessment.

Summary

This data provides the first clear indication of the expected levels of serum tryptase in a large cohort of suspected perioperative anaphylaxis, utilising the only routinely available assay for total tryptase. UK NEQAS External quality assessment data suggest that the majority of participants have assay performance readily able to detect small increments reliably (UK NEQAS Tryptase scheme reports www.immqas.org.uk) meaning that the conclusions are readily translatable to other centres provided laboratories extend their internal controls to reliably report below 3μg/L.

Conflict of Interest: None

Figure legends:

Figure 1: Larger Tryptase changes are seen in higher grades of reaction but overall correlation is poor

Data includes 1746 graded reactions and 307 reactions labelled anaphylaxis (grade uncertain).
Data is presented as Box and Whisker plots with medians (box shoulders = 95% Confidence intervals of median), quartiles (outer box represents 25th and 75th centiles) and max/min ranges.

Peak tryptase and peak to trough increment medians for reaction grades 3, 4& 5 were all statistically different from grade 1 dataset (Kruskall-Wallis $p<0.0001$).

Figure 2: There is a trend to higher peak UMH with higher grades of reaction but overall correlation is poor.

Anaphylaxis ungraded = referral stated "anaphylaxis" but without sufficient details for accurate grading.
Data is presented as Box and Whisker plots) with medians (box shoulders = 95% Confidence intervals), quartiles (outer box represent 25th and 75th centiles and max/min ranges.

Table 1: Incremental changes are of most use within the reference range.

A combination of raised tryptases and incremental changes within then reference range is best. 20% changes are more common within the reference range than 3μg/L increments within the reference range, but 5-10% of the raised tryptases do not change by these thresholds.

ΔTp >20% = positive change in tryptase from trough to peak of >=20%

ΔTp>3μg/L = change in tryptase level from trough to peak of >=3μg/L

*21 (0.6%) additional cases where all tryptases were >=200μg/L (without any measurable increment) could be identified. 117/1053 (11%) samples Tp<5μg/L have ΔTp >=100%

ΔTp>3μg/L is rare below a peak tryptase of 5μg/L, but changes of 20% are common although most are <100%. 43 of 1053 samples (4%) changed between 20 and 100% in low level samples below Tp =5 μg/L.

	ΔTp>3μg/L	*ΔTp >20%*
All Tp (Peak Tryptase) (n=3400)	1762 (52%)*	2750 (81%)
Tp<5 (n=1053)	14 (1.4%)	690 (66%)
4.9<Tp<14 (n=952)	418 (44%)	743 (78%)
Tp>=14 n=1395	1330* (95%)	1316 (94%)
4.9<Tp<11.4 (n=799)	294 (37%)	664 (83%)
Tp>=11.4 (n=1548)	1452* (94%)	1395 (90%)

Table 2: Stratified combinations of thresholds may improve detection within the reference range

Large changes in tryptase (>100%) below a peak tryptase of 5μg/L are rare.

Enforcing a requirement for a 100% change for tryptases below 5μg/L reduces the sensitivity of a 20% threshold but makes little difference to a 3μg/L strategy.

Further studies will be required to determine the clinical predictive values of each strategy in future studies using definitive structured clinical criteria to establish evidence of acute tryptase release

	ΔTp>3μg/L	ΔTp >20%
All Tp levels n=3400	1762 (52%)*	2750 (81%)
Tp>=14 + ΔTp between 5-14 + ΔTp>100%at <5	1930 (57%)	2256 (66%)
Tp>=11.4 + ΔTp between 5-11.4 + ΔTp>100% at < 5	1959 (58%)	2329 (69%)

Table 3: Incremental Tryptase changes within the reference range versus reaction grade have similar utility independent of the upper limit of the reference range used.

Table 3a: Using 11.4 (95th Centile) as upper limit of reference range.

		Tp<11.4 (n=946)		Tp>11.4 (n=800)	
n	Reaction Grade	ΔTp>3μg/L	ΔTp>20%	ΔTp>3μg/L	ΔTp>20%
307	"Anaphylaxis"	29/146 (20%)	68/146 (47%)	149/164 (91%)	118/164 (72%)
230	1	32/179 (18%)	77/179 (43%)	47/51 (92%)	38/51 (76%)
259	2	21/199 (13%)	91/199 (46%)	54/60 (83%)	52/60 (87%)
575	3	64/298 (24%)	131/298 (44%)	270/277 (98%)	203/277 (73%)
641	4	37/249 (15%)	111/249 (46%)	377/391 (96%)	281/391 (72%)
41	5	5/20 (25%)	10/20 (50%)	20/21 (95%)	18/21 (86%)

Table 3b: Using 14 (99th Centile) as upper limit of reference range.

n	Reaction Grade	Tp<14 (n=1014)		Tp>14 (n=732)	
		ΔTp>3μg/L	ΔTp>20%	ΔTp>3μg/L	ΔTp>20%
307	"Anaphylaxis"	43/161 (27%)	78/146 (48%)	135/143 (94%)	118/143 (72%)
230	1	43/192 (22%)	87/192 (45%)	36/38 (96%)	28/38 (74%)
259	2	24/206 (12%)	102/206 (50%)	51/53 (96%)	47/53 (89%)
575	3	93/330 (26%)	133/330 (40%)	236/245 (98%)	200/245 (82%)
641	4	51/265 (19%)	123/265 (46%)	364/375 (97%)	269/375 (72%)
41	5	7/22 (32%)	13/22 (59%)	18/19 (95%)	18/19 (84%)

20% Tryptase increments are more sensitive in the normal range, but less useful in raised tryptases than a 3μg/L increment. Most rises in tryptase below a Tp of 5□g/L are above 20% but less than 100%. Changes in tryptase below 5μg/L are rarely above 100% and are similar for all grades of reaction at 4% (19/543)

Table 4: Urinary methyl histamine identifies potential Mast cell mediator release in a further 3% (100) of cases.

Number of cases	Reaction Grade	UMH>=25μg/mmol* (any sample)	% UMH Positive
18	"anaphylaxis"	11	61
48	1	22	46
45	2	19	45
86	3	56	65
151	4	102	68
7	5	3	43

*UMH >25 μg/mmol was found in 102 cases where ΔTp <3μg/L and 120 cases where ΔTp <5μg/L and 100 cases. In all these cases no incremental tryptase changes are detected using the 3μg/L or 20% incremental thresholds.

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