

Adherence to recommended UK sampling protocols for detecting mast cell mediator release in perioperative anaphylaxis

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ABSTRACT

Background Sheffield National Adverse Reactions Consultancy Service (NARCOS) investigates and triages suspected perioperative anaesthetic reactions to allergy clinics, using serial tryptase samples, urinary methylhistamine and clinical information on a pro forma request.

Objective To determine if current UK guidelines on serial tryptase samples are achieved and describe the patterns of tryptase release.

Method A retrospective review of 3455 NARCOS cases. Tryptase and clinical details were analysed. 1746 had sufficient clinical information to grade the reactions according to the Ring and Messmer scale.

Results 98% provided two or more acute samples, but only 60% supplied clinical information. 308 patients never dropped within the reference range over three samples.

Conclusions Good compliance with UK guidelines for tryptase measurements is achievable in this long-term cohort, but obtaining clinical details at referral remains a challenge. Sample sequence labelling and apparent timing may be misleading. Baseline tryptase may frequently need to be rechecked in allergy clinics to estimate true peak-to-trough changes.

INTRODUCTION

The optimum number of tryptase measurements and the best interpretative strategy for tryptase with robust predictive values have not yet been validated in large cohorts. Current UK guidelines mandate serial samples; two in the acute aftermath plus one 'baseline' sample at 24 hours or more later, to estimate the likely peak tryptase change British Society For Allergy and Clinical Immunology (BSACI) guidelines,¹ Association of Anaesthetists of Great Britain and Ireland (AAGBI) Guidelines 2009² and National Institute for Health and Clinical Excellence (NICE)).³ There remains a lack of consensus that three samples are routinely achievable and some take the view that two are all that is practicable and necessary. Previous reports have shown poor adherence with tryptase sampling.^{4 5} Furthermore, allergy clinical assessment is essential and must include review of the records to confirm the likely diagnosis, investigate and advise on cross-reactivity and future avoidance.

Current UK guidance emphasises that at least two tryptase samples should be obtained postoperatively plus a baseline, but compliance has been very poor in some reports.^{4 5}

We examined 3455 cases in National Adverse Reactions Consultancy Service (NARCOS), a service for the laboratory investigation of adverse

anaesthetic drug reactions, based in Sheffield Teaching Hospitals. We evaluated the compliance with recent UK guidance for tryptase sampling over 17 years, most of which preceded national guidance. NARCOS has always recommended a three-sample strategy and a pro forma set of clinical information, similar in effect to the newer guidance.

METHODS

A retrospective survey of the NARCOS database containing the laboratory information on all samples referred to Sheffield for advice, including tryptase (T) blood tests taken at the time of reaction and 24 hours later. Our original pro forma protocol advised taking serial tryptase measurements; immediately, at 1–6 hours after reaction and at 24 hours. Subsequently, we have promoted similar national guidelines when available. Most patients had at least two serial tryptases (3400/3455), but there was likely to be a wide variation in actual timing, with potential inaccuracies introduced in some during sample labelling and laboratory sample handing and referral onwards. No assumptions were made about the accuracy of the sample timing and most claimed to have provided baseline samples for T3. Samples are therefore analysed according to their labelled sequence as T1, T2 or T3 (first, second and third tryptase samples).

During the time of the analysis, a single tryptase (UniCAB, Thermo Scientific, Uppsala, Sweden) was in use. Statistical and graphical analyses were performed using Analyse-IT software V4.0 (Analyse-IT software, Leeds, UK). Wilcoxon median differences were used to compare the medians. Median and 95% CIs are plotted because the data are not normally distributed.

A 99th centile threshold for determining raised tryptase (14 µg/L) was used. This was locally validated and consistent with the kit 95th centile of 11.4 µg/L and a quoted 'normal' range of 1–15 µg/L (<http://www.phadia.com>).

Reported clinical features were compared with the Ring and Messmer classification (table 1) to assign a grade, where possible.^{5 6}

RESULTS

Raised tryptase is equally common in males and females with suspected perioperative anaphylaxis

Three thousand four hundred and fifty-five cases were referred between January 1998 and May 2015 (1271 males and 2184 females). Twelve cases were excluded because the laboratory failed to send

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Table 1 Clinical grading criterion (modified Ring and Messmer)

	Grade 1 (any severity)	Grade 2 (measurable but not life threatening)	Grade 3 (potentially life threatening)	Grade 4 (definitely life threatening)	'Grade 5'
Skin (not mandatory for grades 2–5)	Any of: itching, flushing, hives or swellings	As grade 1 (but may be absent)	As grade 1 (but may be absent)	As grade 1 (but may be absent)	As grade 1 (but may be absent)
Abdominal (not mandatory for grades 2–5)	None	Minor symptoms	Moderate-to-severe symptoms (eg, vomiting/diarrhoea)	As grade 2 or 3	
Respiratory (asthma, obstruction)	None	Minor/moderate respiratory symptoms	Moderate respiratory compromise	Severe respiratory compromise (arrest)	Fatal
Cardiovascular	None	Minor/moderate hypotension or tachycardia	Moderate/severe hypotension	Severe cardiovascular symptoms (arrest)*	Fatal

Two or more features were required for grading at levels 2–4.

*Unexplained cardiovascular arrest or severe life-threatening hypotension alone was graded as grade 4, even in the absence of respiratory or other symptoms and represented 34% of the grade 4 cohort and 12% of the total evaluable reactions.

any accompanying samples. Forty-three cases had single tryptase measurement only and were excluded from the dataset.

Most clinical services are able to provide at least two tryptases with 98% compliance with minimum UK guidelines and NARCOS requirements

Three thousand four hundred samples had two or more tryptase samples (98% compliance); 71% (2410) had a three tryptase series; 29% (990) had only two tryptases performed. Median levels were significantly different between sample T1 and T2 to T3 ($p < 0.0001$ Wilcoxon median differences).

Tryptase release patterns

All patterns of tryptase changes were apparent. The first tryptase was raised in most cases, but the median peak values are surprisingly low. Approximately 0.5% appeared to have a peak tryptase on the third measurement only. Three hundred and eight cases had raised tryptase (above 14) in all three samples. The first raised tryptase was usually the initial sample (T1): 1312 had a raised T1; 92 had a normal T1 but raised second sample (T2) and 8 had a normal T1 and T2 but a raised T3. 1412/3400 (41.5% of the referred samples) had tryptase ≥ 14 on any one of the three samples. A total of 308 samples still had T3 > 14 $\mu\text{g/L}$, representing 22% of the raised tryptase cohort (figure 1) but only a minority of these were apparently static rises (figure 2). Tryptase results are not normally distributed in any reaction class. Median peak (highest) tryptase Tp in the positive cohort was 39.5 $\mu\text{g/L}$ (IQR 22.6–39.5). In those cases where T3 was ≥ 14 $\mu\text{g/L}$ the median tryptases were higher than in the raised cohort overall. Only 232 Tp were very high at > 100 $\mu\text{g/L}$ and only 2% (82) had Tp > 200 $\mu\text{g/L}$. Tryptase rises were up to 8400% where dilutions were used to estimate peak tryptase, but the dataset only reports maximum results of 200, as this is the routine upper limit of reporting.

Reaction severity

Two thousand and fifty three (60%) of referrals provided some clinical information, in contrast to 98% providing three samples (figure 3); 1746 referrals gave sufficient clinical information to grade the reaction according to the Ring and Messmer scale; a

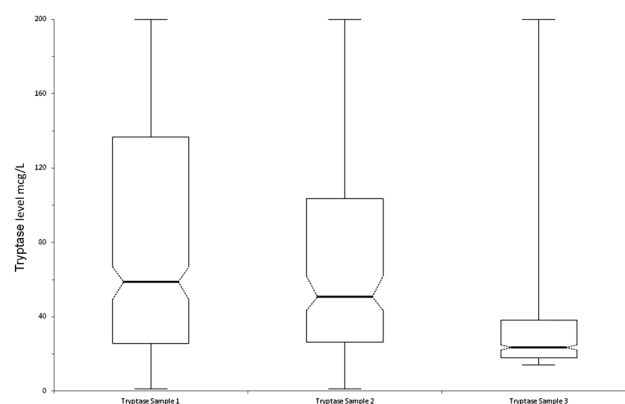


Figure 1 Tryptase levels at each grade. Median values for 308 cases where the third tryptases remained high and above 14 are shown. The medians show a statistically significant downward trend between T2 and T3, consistent with acute mediator release. Median with 95% confidence limits indicated as V-shaped shoulders. Box limits represent 25th and 75th centiles (second and third quartiles). Whiskers represent the untrimmed range of tryptase values.

further 307 specified anaphylaxis but without sufficient details to accurately grade on supplied information.^{5 6}

Two hundred and thirty (13%) of 1746 requests were grade 1 reactions (Ring and Messmer); 259 (15%) were grade 2; (33%) were grade 3; (37%) were grade 4 reactions, of whom 217 (12%) presented solely with severe hypotension/arrest. Forty-one fatal 'grade 5' cases (2.4%) were declared. A single known mastocytosis case was identified at referral. We can extrapolate that >70% of the entire cohort of referrals would

be grade 3–5 reactions, suggesting appropriate pretest selection for suspicion of anaphylaxis.

DISCUSSION

Various UK guidelines have been written to harmonise the evaluation of Allergic reactions to determine the presence of anaphylaxis (AAGBI,² BSACI,¹ NICE).³ Tryptase measurement is an important part of the overall assessment. Recent UK data suggests that compliance with sampling requirements can be poor.^{4 5} Some experts believe that two samples are sufficient to investigate suspected allergic reactions and that compliance with a three-sample protocol may be less effective on a practical level. This study shows that excellent compliance with a three-sample series can be achieved long term, at least in UK health-care systems, although much poorer compliance has been reported in the past.

The role of tryptase measurement in NARCOS is to identify those patients with serial tryptase changes that could represent mast cell mediator release, as a part of their assessment in conjunction with the clinical details and an imputability assessment for potential causes. Interpretation of tryptase changes is not harmonised and there are multiple proposed strategies, but it is generally accepted that some patients with anaphylaxis do not display obvious tryptase release. Positivity can vary from 30% to 60% in published series and may differ depending on the trigger (food, drug), route of administration or severity, and there are no studies using harmonised interpretation criterion that can robustly validate translatable positive or negative predictive values to date. Recently, there has been interest in improving detection of tryptase release at low levels using sensitive increments, including changes as small as 20% within the reference range. AAGBI guidelines recommend that all cases of suspected perioperative anaphylaxis should be assessed in a specialist clinic, to provide confirmatory testing, exclude mimics of anaphylaxis, risk factors such as mastocytosis, identify safe alternatives and confirm baseline tryptase levels where needed.

This large case series suggests that collection of three tryptase samples is useful, and may often be necessary due to differences in the precise timing of samples, inaccurate labelling and potential differences in tryptase release and elimination. Twenty-two per cent of the cases with raised tryptase still had a tryptase above the reference range by the third sample. Some third

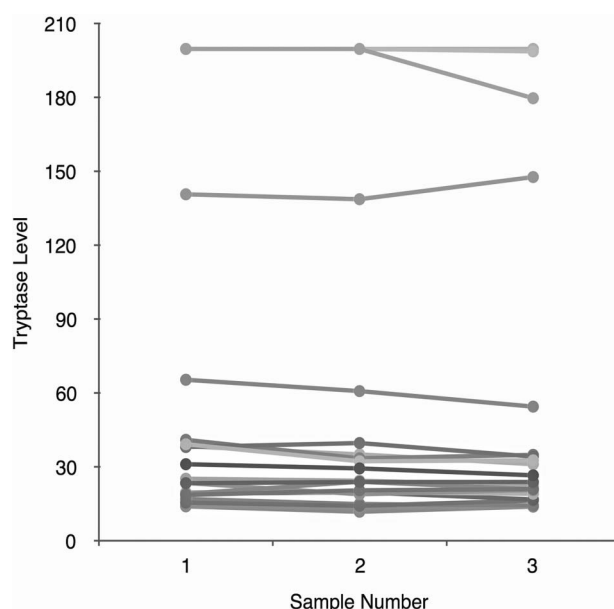


Figure 2 Persistently raised tryptase levels were seen across all three samples in 308/1412 positive cases investigated. One thousand four hundred and twelve cases had at least one raised tryptase. Three hundred and eight of these had raised tryptase in all three sample series. Twenty-nine of these had no measurable change (<20%) across the series to confirm acute phase release of mast cell tryptase. These persistently raised samples might represent mastocytosis or variants, unexplained asymptomatic raised tryptase baselines, inadequate labelling of sample sequence or inappropriately early timing. The majority of cases where the T3 sample was raised showed clear evidence of an acute release curve. Repeat baseline sampling is

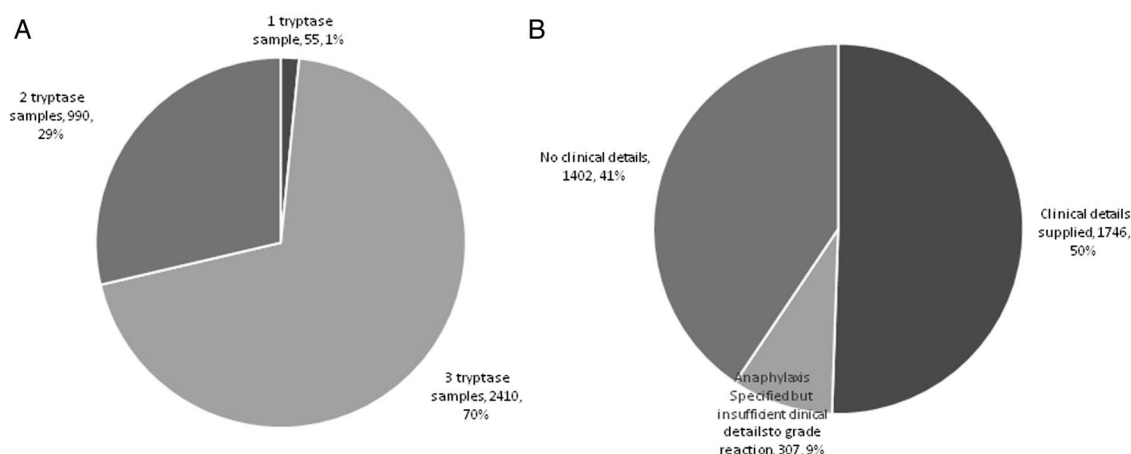


Figure 3 Compliance with sample guidelines and provision of clinical details. (A) Number of serial tryptase samples provided. (B) Provision of adequate clinical details with samples to grade reaction. Provision of adequate clinical information to enable accurate reaction grading is only provided by half of the referring laboratories. Provision of adequate samples compliant with current guidance is consistently excellent in this cohort over a 17-year period.

Short report

samples may have been taken at <24 hours, or tryptase had not reached baseline by the next day. Evidence of early sampling (where all three samples were definitely taken within the 12 hours following the reaction) was seen only 8 of 29 persistently raised sample series; 10/29 were however definitively labelled as >24 hours afterwards, 3/29 between 16 and 24 and the remainder were ambiguously labelled (although they mostly appeared to be '≥24-hour' samples).

Despite the recommendations on sample timing, the reality in a busy clinical unit is that samples will be taken when possible and convenient.

In a few cases (0.5%), tryptase release was only evident on the third sample. This could have many causes including sample mislabelling, analytical errors in sample processing and reporting. These are not uncommon in clinical scenarios. Irrespective of the cause, and it remains an important observation that potentially impacts on the performance of the screening strategy.

Baseline sampling may well occur during routine venesection rounds at fixed times, such that the third sample may well be taken well before 24 hours has elapsed, or long after. This, combined with variation in tryptase release and clearance, prevents any robust estimation of true peak tryptases. The maximum value seen cannot be assumed to be near the true 'peak'. It may be taken on the increasing or the decreasing part of the curve. This is at least one potential reason why correlation between reaction severity and apparent peak tryptase is suboptimal overall, and unreliable on an individual basis.

Our 17-year experience validates the practicality of good compliance with current NICE recommendations³ that at least two tryptase samples should be taken in the aftermath of a reaction (plus a baseline sample), but this dataset suggests that the baseline may have to be later than 24 hours from the reaction and may always need to be rechecked during clinic assessment some weeks later to establish a true baseline. Compliance with adequate sampling is possible over the longer term even though other UK centres find that adherence is much worse. The only obvious difference is the ready availability of clinical advice at referral, and a written protocol and form for investigation and submission of cases. In addition, where possible, further information is sought when needed from the referrer.

Persistently raised tryptase across the three samples is surprisingly common in this series and 308 patients had persistently raised tryptase, out of 1412 patients who showed a raised tryptase in at least one of the three samples. Within this persistently raised group all patterns of changes could be seen. Stably raised tryptase without evidence of acute changes of >20% was quite unusual. Only 29 patients showed an unmeasurable change (<20%) across the samples and therefore could potentially represent mastocytosis or some other form of chronic tryptase elevation; however, for eight of these, the T3 sample was taken <12 hours after the event, but 10 were clearly labelled as 24 hours or later. The baseline in these cases is therefore unknown. However, the majority of the cohort of 1412 tryptase-positive patients had dropped to within the reference range by sample 3. By the same logic, it cannot be assumed that the third sample is indeed the baseline level. This suggests considerable variation in tryptase kinetics postreaction in some cases, as previously noted in the early literature.⁷

We report long-term successful receipt of multiple tryptase samples in our service which may not be typical of the UK experience. Other services report that sample provision has been poor.⁵ Therefore, we demonstrate that it can be done over a long period of time, but we can only speculate on the reasons for the success. Our referring anaesthetists are provided with clear guidance on sampling through a structured referral template and ready access to advice. Furthermore, much credit for ensuring adequate sampling may lie with the local laboratories that collect and triage the samples to us. With a compliance rate of 98% for provision of serial tryptase measurement, this report suggests that the provision of a structured referral template (such as the AAGBI referral document), requesting both clinical details/operative record and serial timed samples at the point of referral, may improve compliance with national sampling recommendations in conjunction with ready access to telephone advice. This is not the only reason, however, as many anaesthetists refer in without using the template itself, although many provide the information required. UK Anaesthetists are therefore capable of excellent adherence to sample guidance over a prolonged period of time, but obtaining full clinical details at the time of referral may be more challenging, since only 60% of referrers provided adequate clinical information to grade the reaction. In mitigation, it is impossible to know if clinical information was provided to the local referring laboratory, but not passed on, as the samples are usually forwarded by the local laboratory rather than the clinical service. Further allergy clinic referral is always necessary to complete the investigation, risk assess and advise on safe alternatives.

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Contributors WE designed the study, performed the analysis and authored the paper.

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Data sharing statement The NARCOS dataset continues to be populated and is being analysed inhouse for further analysis and publication.

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