Editorial

Laboratory investigation of anaphylaxis: not as easy as it seems

Anaphylaxis and anaesthesia

Anaphylaxis is one of the most serious complications of anaesthesia. It has an incidence of approximately 1:10 000 to 1:11 000 anaesthetics, and is more common in women compared with men (1.55 per 10 000 versus 0.55 per 10 000 anaesthetics, respectively) [1]. It occurs with sufficient frequency that every anaesthetist is likely to experience at least one episode during their career. The most common drugs to cause peri-operative IgE-mediated anaphylaxis are the neuromuscular blocking drugs, with atracurium, suxamethonium and rocuronium implicated most frequently. In one very recent UK series, these drugs accounted for 38% of the reactions, followed by antibiotics at 8%. Interestingly, chlorhexidine accounted for 5% of reactions, and patent blue dye for 6% [2]. Mortality from anaesthesia-related anaphylaxis is reported to be between 1.4% and 9% [3–5], and in the case of reactions to neuromuscular blocking drugs has been associated with male sex and a history of cardiovascular disease, including hypertension and ongoing beta-blocker treatment. The persisting relatively high mortality despite following international guidelines is suggested as reflecting the severity of reactions to these agents [4].

A UK-wide study of anaphylaxis during anaesthesia is due to start in the near future [6], so it is timely to remind ourselves of some of the basic information and the value of laboratory testing.

The symptoms of anaphylaxis – e.g. hypotension, respiratory failure, laryngeal oedema, asthma, urticaria, erythema and angioedema – can be mediated via a number of mechanisms. Reactions mediated by IgE occur when a patient makes antibodies with specificity for an antigen, such as the anaesthetic drug. Similar symptoms can be generated via other mechanisms. For example, reactions to neuromuscular blocking drugs can be associated with exposure to quarternary ammonium ions in other compounds, such as over-the-counter remedies and cosmetics: in Norway, cough syrups containing pholcodine have been implicated – and indeed, withdrawn from sale, although they are still available in chemists in the UK [7]. The IgE antibodies bind to mast cells (and basophils) via high-affinity IgE receptors; when the patient re-encounters the anaesthetic drug, cross-linking of the IgE receptors causes activation of the mast cells, with release of powerful, preformed, granule-derived mediators including histamine, proteoglycans and the neutral proteases tryptase and chymase. Newly formed mediators, e.g. prostaglandins, leucotrienes and platelet activation factor, are also released. Together, these inflammatory mediators act either locally or systemically to cause capillary leakage, mucosal oedema and smooth muscle contraction, generating the symptoms observed [8]. Mast cells are found in all vascularised tissues but with higher numbers strategically located at host/environment interfaces i.e. skin and mucosal surfaces of the respiratory and gastrointestinal tracts. They exhibit different phenotypes, defined according to their secretion of tryptase and chymase and their homing site, with the number and phenotype of mast cells changing during pathological processes. It is important to note that mast cells are also found in normal and diseased human heart tissue, in close contact with the blood vessels [9, 10].

Mast cells (and basophils) can be activated directly, causing the same mediator release but without the specific interaction of IgE antibodies. The term ‘anaphylaxis’ is now used to describe both IgE and non-IgE mediated reactions; the subdivision into allergic or non-allergic anaphylaxis occurs only after diagnostic testing shows the underlying cause or mechanism [11]. The term ‘anaphylactoid’ is no longer used.

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Guidelines on the management of suspected anaphylaxis have been published by the Association of Anaesthetists of Great Britain and Ireland [12], and the British Society for Allergy & Clinical Immunology published guidelines focused on the allergist's role in investigating suspected anaphylaxis during anaesthesia [13]. More recently, the National Institute of Health and Care Excellence has published guidelines on the diagnosis and manangement of drug allergy in adults [14]. These documents all highlight the importance of serum tryptase measurements in the immediate investigation of patients with suspected anaphylaxis. However, considering the widespread agreement about the use of tryptase measurements among anaesthetists, allergist and immunologists, it is surprising that it is so rarely requested and the interpretation of results is so poorly understood. This editorial aims to highlight the value of serum tryptase measurements and some of the pitfalls in the interpretation of results.

Tryptase

Tryptase is a serine peptidase enzyme, made and stored in mast cells and basophils irrespective of tissue location. There are two forms of tryptase, designated α and β. α-Tryptase is not stored in the mast cell secretory granules and is constantly being released in small amounts, along with pro-β-tryptase; hence tryptase is detectable in normal plasma [15]. High concentrations of α-tryptase are seen in systemic mastocytosis (the accumulation of mast cells in multiple organs including skin, bone marrow, liver and gastrointestinal tract, causing flushing, pruritus, osteoporosis, anaemia and occasionally anaphylactic symptoms) [15]. Mature β-tryptase is a catalytically active, heparin-stabilised tetramer that is stored within the mast cell secretory granule, along with other preformed mediators [16]. Anaphylaxis is associated with high concentrations of β-tryptase, although the role of tryptase in the pathogenesis of anaphylaxis is poorly defined, with limited evidence for its involvement in the clinical symptoms. However, it has been suggested that tryptase can increase bronchoconstriction [17] and can interact with the complement and clotting cascades, e.g. in inactivating pro-coagulant proteins and promoting fibrin clot lysis [18].

The reference range for serum tryptase is in the order of 2-14 $\mu g.l^{-1}$, although each laboratory or method should have established or verified its quoted reference range. Tryptase concentrations in serum or plasma are raised in most subjects with systemic anaphylaxis that is severe enough to cause hypotension [19]. The serum tryptase concentration peaks between 15 and 120 minutes after encountering the relevant antigen/allergen, with both the timing and the peak value dependant on both the nature and route of the stimulus, and the clinical severity [15]. Tryptase is catabolised by the liver, with a half-life in vivo of approximately 2.5 hours. It is occasionally suggested that histamine is measured in the investigation of anaphylaxis but the half-life of histamine is so short (approximately 2-3 minutes) that this is of no practical value [8].

Tryptase is a relatively stable protein in vitro; it is therefore surprising that some laboratories refuse to measure tryptase concentrations unless the sample has been stored frozen. Blood can be collected into EDTA or heparin tubes, or tubes with no anticoagulant, and should be separated from the cells on receipt in the laboratory. It is preferable that the samples be analysed rapidly e.g. within 5–7 days of being taken, and the sample *may* be frozen if there is going to be a delay in analysis. However, tryptase is sufficiently stable for samples to be transported at room temperature (e.g. by first class post) if they are being sent between laboratories [20]. The laboratory measurement is straightforward, using an automated immunoassay system (the Phadia™ system from Thermo Fisher, Scientific Waltham, MA USA) is the most commonly used), and detects both α- and β-tryptase. Laboratories measuring serum tryptase should validate the assays using internal quality control material and participate in an external quality assurance scheme e.g. United Kingdom National External Quality Assurance Scheme (UKNEQAS).

Investigation of anaphylaxis

The guidelines on the investigation of suspected anaphylaxis suggest that samples, labelled to include the date and time, be taken for serum tryptase measurement as soon as feasible after resuscitation has started, at 1-2 hours after the onset of symptoms, and either 24 hours

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after the reaction or in convalescence [12–14]. Scientifically, this is appropriate, but practical experience suggests that these guidelines are not adhered to; laboratories rarely receive a sensible series of samples, and frequently receive samples that show no date or time, with sometimes limited patient-identifying information. There is a narrow window of opportunity in which to analyse these samples, and if they are not collected at the time, it is impossible to gather that information without significant risk to the patient. Understandably, patients who have had anaphylaxis may be cared for by various teams and are often transferred e.g. from operating theatre, to recovery, to the intensive care unit and then to the ward; 'handing over' which samples need to be taken may not be the highest priority. A sensible suggestion is to request more samples, for example as soon as possible after the onset of the reaction then at approximately 1, 2, 3, 6, 12 and 24 hours post-reaction. This total number of samples is rarely received, but such a request does encourage samples to be taken after the acute episode has resolved, and highlights that even if the two-hour sample has been missed, others can be taken later. There are some additional advantages to having a greater number of samples to analyse; it is very reassuring to see the tryptase concentration peak and then fall within its expected half-life. The amount of fluid given rapidly to a patient during/after anaphylaxis may cause haemodilution, and in some cases, the tryptase concentration can be rather variable in the few hours; more results are easier to interpret. Finally, should the patient not recover and the case be referred to the Coroner, a series of two or three tryptase results that are consistent with each other gives greater confidence in the results and their interpretation.

Serum tryptase concentration can also be raised in systemic mastocytosis, some myelodysplastic syndromes, mast cell leukaemia and end-stage renal failure, hence the importance of checking a sample 'in convalescence' to confirm that the tryptase concentration has returned to a normal or near-normal level.

A peak serum tryptase concentration of $\geq 50\ \mu g.l^{-1}$, taken from a patient with relevant symptoms, would be consistent with IgE-mediated anaphylaxis, particularly if the tryptase concentrations then fall to normal in convalescence. Non-IgE mediated reactions, e.g. direct mast cell activation or complement activation, can also generate raised tryptase concentrations; these are typically lower values, between 20 and 50 $\mu g.l^{-1}$ [20]. Laboratories should add interpretative comments to the serum tryptase results but in all situations, the result must be interpreted in the context of the time of the suspected reaction, the course of the reaction, the time of the sample(s) and any fluids that may have been given during resuscitation.

The investigation of patients after the anaphylaxis has been successfully treated should be done in specialist allergy centres with the appropriate expertise, and is well described by Ewan et al. [13]. There are, however, situations where the patient does not recover. Irrespective of the samples that may have been taken before death, tryptase concentrations can also be measured in the blood post mortem.

The site of sampling for tryptase measurements post mortem is important because in individual patients, there may be marked differences between the tryptase results in samples from the aorta, subclavian artery and femoral artery [21]. Samples taken post mortem are often haemolysed, but high concentrations of haemoglobin in itself do not seem to influence the analysis of tryptase [21]. The haemolysis may be a surrogate indicator of cell autolysis and liquefaction in the tissues and blood vessel walls, and in areas with a high number of mast cells (e.g. respiratory tract and heart). This cell lysis may in itself increase the release of tryptase. It is also possible that release of tryptase from mast cells in the respiratory system or heart is exacerbated by prolonged cardiac massage or defibrillation. It is preferable, therefore, that post-mortem samples for tryptase are taken from femoral blood vessels; in any case, the site of sampling should be specified on the request form.

Tryptase concentrations in samples taken post mortem from patients with 'non-anaphylactic' causes of death show a much wider range, and are higher than in samples taken ante mortem [21]. Deaths from multiple trauma are associated with higher tryptase concentrations post mortem than in deaths from non-traumatic or single trauma causes [22]. The tryptase reference ranges for live subjects (2-14 $\mu g.l^{-1}$ indicating normal, and $> 50\ \mu g.l^{-1}$

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consistent with anaphylaxis) are not applicable for samples taken post mortem. McLean-Tooke et al. constructed a receiver operating characteristic (ROC) curve for post-mortem aortic tryptase concentration for the diagnosis of anaphylactic-associated death, concluding that a cut-off concentration of 110 $\mu g.l^{-1}$ gave the best specificity and sensitivity. They do, however, highlight that this concentration, and even a cut-off value of 50 $\mu g.l^{-1}$, would have missed two cases of (food related) anaphylaxis [21]. Considering the limited literature, and the complexity of interpretation, we would suggest an arbitrary cut-off of 100 $\mu g.l^{-1}$, with values below this not being *suggestive* of anaphylaxis contributing to or causing death. However, it is vital to report the possible reasons for the raised tryptase concentration, e.g. sample degradation, release secondary to the resuscitation process (particularly cardiac defibrillation) or a non-immune mechanism. The interpretation of these results must factor in the time of death with respect to the suspected reaction in addition to the course of the reaction, the time of the sample(s) and any fluids that may have been used during resuscitation.

Conclusions

The laboratory measurement of tryptase concentration is a very valuable tool to support the diagnosis of anaphylaxis, both in patients who recover and in those who do not. Unfortunately, interpretation of tryptase results can be complicated by the very nature of the symptoms and the large volumes of fluid that may be given, the use of cardiac massage and defibrillation, and the inherent characteristics of tryptase. The anaesthetists must accept responsibility for taking, labelling (and it is vital to include the date, time and clinical details) and sending the samples [12]. The laboratories need to be aware that tryptase is stable, that the sample does not need to be frozen, and that tryptase can be measured in samples collected into most normal blood collection tubes – most patients who have had anaphylaxis are likely to have had blood taken for urea/electrolytes and a full blood count! The interpretation of results is not simply a question of: "is the patient's value above or below the reference range?" It is impossible to say that a tryptase above a certain concentration definitely indicates anaphylaxis, and that a normal tryptase definitely rules it out. However, using the clinical picture and the tryptase results, we should be able to give an interpretation based on data and the 'balance of probability'.

Competing interests

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Editorial

MBRRACE-UK – the new home for the Confidential Enquiries into Maternal Deaths – reports for the first time

The Confidential Enquiries into Maternal Deaths (CEMD) in the UK has been revitalised with the publication of a new report [1]. The first CEMD Report, covering maternal deaths in England and Wales during 1952-54, was published in 1957 [2], and the process operated continuously until the publication of the last report, 2006-08, in 2011 [3]. However, soon after the laudatory retrospective of a half-century of continuous data collection and resultant systems changes [2], the chain was broken. A competitive tendering process for the right to run the CEMD was held in 2010, resulting in the award of the contract to a new consortium: Mothers and Babies – Reducing Risk through Audits and Confidential Enquiries across the UK (MBRRACE-UK). Before it could start, but after the programmed demise of the Centre for Maternal and Child Enquiries (CMACE, which had been running the Enquiries previously) in 2011, a review panel was instructed to examine the requirements for the programme [4]. The review panel concluded that the maternal and infant enquiries should continue, and MBRRACE-UK was reconfirmed in its status with a start date of May 2012 [1]. This confused period led to a breakdown in the established reporting and note review systems, with a danger that cases would be lost. Incomplete ascertainment of maternal deaths might look good for comparisons – UK mortality rates might become better than Albania's [5]! – but does not square with the CEMD ethos of complete thoroughness and honesty. Reassuringly, MBRRACE-UK has obtained all case records from 2009 onwards and is confident that there are no 'missing' maternal notes. For the first time, the Report also includes cases from Ireland as part of a joint Confidential Enquiry process. The good news is that there has been a statistically significant decline in the maternal mortality rate, principally due to a decline in mortality from direct (obstetric) causes.

The format of this and future CEMD reports will be different to

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