

EDITORIAL

Clinical & Experimental Allergy

Laboratory diagnosis of acute anaphylaxis*

*This editorial discusses the findings of the paper in this issue by M. Borer-Reinhold et al. [14], pp. 1777–1783.

S. G. A. Brown^{1,2} and S. F. Stone²

¹Emergency Department, Royal Perth Hospital, and ²Centre for Clinical Research in Emergency Medicine, Western Australian Institute for Medical Research and the University of Western Australia, Perth, WA, Australia

Anaphylaxis has been termed the ‘quintessence of emergency medicine’ [1], occurring unexpectedly and progressing rapidly with sometimes fatal consequences if unrecognized and not treated immediately with adrenaline and fluid resuscitation. Clinical diagnosis is usually straightforward for experienced emergency physicians and anaesthetists, who may deal with anaphylaxis frequently and/or regularly prepare themselves for this emergency.

Nevertheless, an inescapable reality is that acute anaphylaxis will be frequently – if not mostly – managed by a range of health care providers who may never have encountered a case before and/or may not be familiar with the subtleties of some modes of presentation and diagnostic pitfalls. Furthermore, ongoing care relies on detective work after the fact. This can be difficult given the incomplete medical notes that often come from busy emergency departments or from solo general practitioners dealing with cases single-handed.

Do we need a laboratory test to diagnose anaphylaxis?

Getting the diagnosis right is important because, once we know what we are treating, efforts at secondary prevention can be made. Further episodes and deaths can be prevented by identifying the likely cause(s) and thus appropriate avoidance strategies, immunotherapy (for insect venom allergy) and provision of an adrenaline auto-injector and an action plan for the patient to follow if another reaction occurs [2].

Even with exemplary clinical assessments, diagnostic issues emerge, for example:

- Diagnostic skin features are absent or very subtle in ~20% of cases [3, 4]. When assessing an acute onset of severe hypotension, collapse or bronchospasm without typical skin features, how do we diagnose or exclude anaphylaxis?
- Bradycardia is a known feature of severe hypotensive anaphylaxis [5]. However, in some cases, clinicians might have difficulty deciding between a diagnosis of severe anaphylaxis versus a faint (vasovagal), perhaps superimposed on an otherwise mild reaction.
- Cardiovascular collapse under anaesthesia with ECG changes; is this anaphylactic hypotension causing impaired coronary perfusion, cardiac anaphylaxis due to a direct mediator effect on the heart (which is loaded with mast cells), or simply an acute coronary event? [6]
- Gastrointestinal features (severe nausea, persistent vomiting and cramping abdominal pain) are important markers of a severe allergic reaction in the right clinical setting [4, 7]. But consider a reaction with relatively minor skin involvement (perhaps subtle flushing), severe abdominal pain, vomiting, incontinence and a borderline blood pressure by the time of reaching medical care. Was this anaphylaxis, food intolerance, scombroid fish poisoning or perhaps a viral illness?

We fondly recall a man referred to one of our snakebite studies. He was gardening and yelled out to his wife ‘I’ve been bitten’, then stopped breathing and had a brief seizure when propped upright in a chair (not the best first aid, as Pumphrey has observed [8]). When the ambulance arrived, he was lying flat but still confused, incontinent, pale and hypotensive (systolic blood pressure 50 mmHg). His blood pressure improved steadily over an hour without any fluids or adrenaline. Australian snakebites can cause syncope, hypotension and severe coagulopathy, so the working diagnosis started as snakebite at the first hospital he was taken to. His clotting study results came

Correspondence:

Prof. Simon G. A. Brown, Centre for Clinical Research in Emergency Medicine, Western Australian Institute for Medical Research, Level 5, MRF Building, Rear 50 Murray St, Perth WA 6000, Australia. E-mail: simon.brown@uwa.edu.au

Cite this as: S. G. A. Brown and S. F. Stone, *Clinical & Experimental Allergy*, 2011 (41) 1660–1662.

back normal and sometime later, after transfer to a tertiary hospital, the diagnosis changed to 'first seizure'. He was discharged home for an outpatient electroencephalogram and neurology follow-up. We disagreed with the medical team on this one, so we sent our study blood samples, collected at several time-points from arrival at the first hospital, for serial tryptase measurements. Tryptase peaked at 18.8 IU/L (laboratory normal range <13) 3 h after the collapse then fell to 10.9 IU/L, confirming our diagnosis of anaphylaxis. He also had high levels of bee venom-specific IgE, so he received bee venom immunotherapy instead of an electroencephalogram!

Tryptase assays

Circulating human tryptases consist mainly of the mature or active β -tryptases and inactive pro-tryptases (α and β). Baseline or 'constitutive' levels of immunoreactive tryptase in serum are thought to consist of the pro- α and pro- β tryptases, whereas mature β -tryptases are released by mast cell degranulation [9]. The only widely available diagnostic tryptase assay, the Phadia ImmunoCAP Tryptase, measures all forms of circulating tryptase. Basal tryptase concentrations measured by the ImmunoCAP for any given individual appear to be very stable over both the short-term (non-reactive individuals during sting challenge) and in the longer term (~3 months) [10].

Following mast cell and basophil degranulation, serum tryptase concentrations reach a peak after 1–2 h [11, 12], however, a single measurement of tryptase has a low sensitivity for a diagnosis of anaphylaxis because the peak of active β -tryptase may be missed or may occur within the normal range for the assay. One solution could be to measure only mature β -tryptases, in which case there would be no normal range – any detectable β -tryptase should be diagnostic of degranulation. Unfortunately, a specific mature β -tryptase assay is not currently available for diagnostic use and a large-scale validation of sensitivity and specificity in a clinical setting is required.

Another potential solution is to measure total tryptase (all forms) at several time-points to identify the downward slope as the reaction resolves (e.g. at first assessment, 1–2 h later and then during convalescence). As well as increasing sensitivity, such an approach increases specificity because high basal tryptase concentrations, due either to individual variation or underlying mastocytosis, will be identified.

Serial measurement of serum tryptase to diagnose anaphylaxis

In a multi-centre Emergency Department (ED) study of moderate and severe anaphylaxis from all causes

(stings, drugs and foods), defined according to NIAID-FAAN criteria [13] and graded according to our previously published system [4], we found the sensitivity of serial tryptase measurement to be 55% and 75% for moderate and severe anaphylaxis, respectively [12]. We defined a positive 'delta-tryptase' as a difference of 2.0 μ g/L or more between high and low values, taken at arrival, 1 h and finally just prior to discharge from the ED. Our approach was based on previous insect sting challenge work, including a receiver–operator characteristic curve analysis with a large number of non-reacting controls where no significant fluctuations in tryptase occurred over time [10].

In this issue, Borer-Reinhold et al. [14] provide further evidence to support use of serial tryptase measurements to diagnose anaphylaxis where it is caused by insect venom. They included mild cases that did not meet the NIAID-FAAN definition of anaphylaxis, used a different severity grading system and had a different approach to defining a positive delta-tryptase than we have done, namely a relative change rather than absolute change. Nevertheless, the results are remarkably consistent with previous work. In theory, their relative delta-bound approach, although more complex, may be more sensitive than the absolute delta-value, particularly for patients with a very low or very high baseline tryptase. Unfortunately, serial measurement of tryptase is likely to have a sensitivity of no more than 80% in severe hypotensive anaphylaxis, and probably much lower for mild and moderate hypersensitivity reactions and for severe reactions that are confined to the airways, when it is probable that less tryptase reaches the circulation. Tryptase also still has a very limited role in post-mortem diagnosis, where tryptase measurement has both poor sensitivity (death may occur before the peak) and poor specificity because non-specific tryptase release occurs during and/or after death [15].

The essence of the problem

A number of mediators are generated during anaphylaxis with redundancy of effects, multiple overlapping pathways and involvement of several cell types. Tryptase is only one of these mediators and it may not be released in large amounts. Furthermore, mediators may not be easily detectable systemically if most of the 'action' is occurring in the airways or gastrointestinal tract. There is much that we do not know about what converts (amplifies) a local reaction into a systemic reaction, thus defining anaphylaxis [16]. Patients also present with different clinical reaction patterns and we do not fully understand why this happens.

Where to from here?

On the basis of currently available evidence we encourage medical staff to take blood for tryptase measurement on ED arrival (or reaction onset), 1 h later and then prior to discharge. In practice, however, this is only done as part of one of our research protocols or where the diagnosis is doubtful and the treating doctor seeks advice from us. Although serial tryptase measurements do not help with initial management, the results can be useful at a later date. A positive delta-tryptase may confirm a diagnosis of anaphylaxis where this is uncertain clinically; however, a negative result will usually be unhelpful because false negatives are common.

Having said this, the evidence for using delta-tryptase to diagnose anaphylaxis is still too limited to make firm recommendations. It would be useful to perform a large, carefully designed prospective multi-centre study that includes insect venom, drug, food and peri-operative anaphylaxis and also controls with other relevant acute illnesses, for example, severe asthma, severe sepsis, acute gastrointestinal illnesses and major trauma. Such a study is required to confirm the high specificity

of delta-tryptase in real life, and to determine the precise effects of pre-test probability (likelihood of anaphylaxis), reaction type, severity and causation on diagnostic performance.

Another question that remains is how often will a laboratory diagnosis change management? Prevention of anaphylaxis is all about risk management and allergists are used to dealing with uncertainties. Even if the diagnosis of anaphylaxis is uncertain or if the reaction was only a mild one not meeting any diagnostic criteria for anaphylaxis, allergists will take reasonable steps to identify potential allergens, to minimize the risks of recurrence and to ensure the patient is prepared for another reaction.

Finally, we need to consider and perhaps evaluate alternative laboratory approaches. These could include highly sensitive assays for mature β -tryptase and 'multi-marker' approaches, analysing a number of mediators simultaneously to improve sensitivity. This approach might also help us to identify different types of anaphylaxis [17]. Ultimately, however, we return to the essence of the problem – we need a better understanding of acute anaphylaxis in humans, specifically the pathways and mediators involved.

References

- 1 Brown AFT. Anaphylaxis: quintessence, quarrels, and quandaries. *Emerg Med J* 2001; 18:328.
- 2 Lieberman P, Kemp SF, Oppenheimer J, Lang DM, Bernstein IL, Nicklas RA, et al. The diagnosis and management of anaphylaxis: an updated practice parameter. *J Allergy Clin Immunol* 2005; 115:S483–523.
- 3 Brown AFT, McKinnon D, Chu K. Emergency department anaphylaxis: a review of 142 patients in a single year. *J Allergy Clin Immunol* 2001; 108:861–6.
- 4 Brown SGA. Clinical features and severity grading of anaphylaxis. *J Allergy Clin Immunol* 2004; 114: 371–6.
- 5 Brown SGA, Blackman KE, Stenlake V, Heddle RJ. Insect sting anaphylaxis: prospective evaluation of treatment with intravenous adrenaline and volume resuscitation. *Emerg Med J* 2004; 21:149–54.
- 6 Brown SGA. The pathophysiology of shock in anaphylaxis. *Immunol Allergy Clin North Am* 2007; 27:165–75.
- 7 Brown SGA, Mullins RJ, Gold MS. Anaphylaxis: diagnosis and management. *Med J Aust* 2006; 185:283–9.
- 8 Pumphrey RS. Fatal posture in anaphylactic shock. *J Allergy Clin Immunol* 2003; 112:451–2.
- 9 Caughey GH. Tryptase genetics and anaphylaxis. *J Allergy Clin Immunol* 2006; 117:1411–4.
- 10 Brown SGA, Blackman KE, Heddle RJ. Can serum mast cell tryptase help diagnose anaphylaxis? *Emerg Med Australas* 2004; 16:120–4.
- 11 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; 83:1551–5.
- 12 Stone SF, Cotterell C, Isbister GK, Holdgate A, Brown SGA. Elevated serum cytokines during human anaphylaxis: identification of potential mediators of acute allergic reactions. *J Allergy Clin Immunol* 2009; 124:786–92 e4.
- 13 Sampson HA, Munoz-Furlong A, Campbell RL et al. Second symposium on the definition and management of anaphylaxis: summary report—Second National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network symposium. *J Allergy Clin Immunol* 2006; 117: 391–7.
- 14 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; 41:1777–83.
- 15 Mayer DE, Krauskopf A, Hemmer W, Moritz K, Jarisch R, Reiter C. Usefulness of post mortem determination of serum tryptase, histamine and diamine oxidase in the diagnosis of fatal anaphylaxis. *Forensic Sci Int* 2011; 212:96–101.
- 16 Golden DB. What is anaphylaxis? *Curr Opin Allergy Clin Immunol* 2007; 7:331–6.
- 17 McCall-Culbreath KD, Li Z, Zhang Z, Lu LX, Orear L, Zutter MM. Selective, α 2 β 1 Integrin-Dependent Secretion of IL-6 by Connective Tissue Mast Cells. *J Innate Immun* 2011; 3:459–70.