Use and interpretation of acute and baseline tryptase in perioperative hypersensitivity and anaphylaxis

Use and interpretation of acute and baseline tryptase in perioperative hypersensitivity and anaphylaxis

Joana Vitte MD, PhD [1,2,3], Vito Sabato MD, PhD [4,5], Charles Tacquard MD[6], Lene H. Garvey MD, PhD [7], Moïse Michel PharmD [1,2,8], Paul-Michel Mertes MD, PhD[6], Didier G. Ebo MD, PhD[4,5], Lawrence B. Schwartz MD, PhD[9] and Mariana C. Castells MD, PhD[10] *

1 Aix-Marseille Univ, IRD, APHM, MEPHI, Marseille, France

2 IHU Méditerranée Infection, Marseille, France

3 IDESP, INSERM UMR UA11, University of Montpellier, Montpellier, France

4 University of Antwerp, Faculty of Medicine and Health Sciences, Department of Immunology, Allergology, Rheumatology and the Infla-Med Centre of Excellence, Antwerp (Belgium) and Immunology, Allergology, Rheumatology, Antwerp University Hospital, Antwerp (Belgium)

5 AZ Jan Palfijn Gent, Department of Immunology and Allergology, Ghent, Belgium

6 Nouvel Hôpital Civil, hôpitaux universitaires de Strasbourg, service d'anesthésie-réanimation chirurgicale, 1, place de l'Hôpital, BP 426, 67091 Strasbourg cedex, France

7 Allergy Clinic, Department of Dermatology and Allergy, Gentofte Hospital, Denmark and Department of Clinical Medicine, University of Copenhagen, Denmark

8 Laboratoire d'Immunologie, CHU de Nîmes, Nîmes, France

9 Department of Internal Medicine, Division of Rheumatology, Allergy & Immunology, Virginia Commonwealth University, Richmond, VA 23298, USA

10 Division of Allergy and Clinical Immunology, Brigham and Women's Hospital, Boston, MA; Harvard Medical School, Boston, MA

***Corresponding author**

Prof Mariana C. Castells, Division of Allergy and Clinical Immunology, Brigham and Women's Hospital, Boston, MA; Harvard Medical School, Boston, MA

mcastells@bwh.harvard.edu

4774 words sections 1-6 (Introduction through Acknowledgments); 5 figures; 124 references

ORCID numbers
Joana Vitte: 0000-0002-3344-9408
Vito Sabato: 0000-0002-1321-314X
Charles Tacquard: 0000-0002-7274-6018
Lene H. Garvey : 0000-0002-7777-4501
Moïse Michel: 0000-0002-0460-9898
Paul-Michel Mertès : 0000-0002-6060-9438
Didier G. Ebo : 0000-0003-0672-7529
Lawrence B. Schwartz : 0000-0002-4379-6729
Mariana C. Castells : 0000-0001-6451-0163

COI

JV reports speaker and consultancy fees in the past 5 years from Meda Pharma (Mylan), Novartis, Sanofi, Thermo Fisher Scientific, outside the submitted work. LHG is a consultant and adjudication committee member for Novo Nordisk A/S, Denmark and Merck, New Jersey, USA. LBS: VCU receives royalties from Thermo Fisher for the tryptase assay that are shared with its inventor, LBS. The other authors declare that they have no COI relevant to this work.

Funding: No specific funding was allocated for this review.

Abstract

Paired acute and baseline serum or plasma tryptase sampling and determination have recently been included as a mechanistic approach in the diagnostic and management guidelines of perioperative immediate hypersensitivity and anaphylaxis. The timing of this paired sampling is clearly defined in international consensus statements, with the optimal window for acute tryptase sampling between 30 minutes and 2 hours after the initiation of symptoms, while baseline tryptase should be measured in a sample collected before the event (pre-op) or at least 24 hours after all signs and symptoms have resolved. A transient elevation of the acute tryptase level greater than [2 + (1.2xbaseline tryptase level)] supports the involvement and activation of mast cells.

Here, we provide the clinical, pathophysiological, and technical rationale for the procedure and interpretation of paired acute and baseline tryptase. Clinical examples, up-to-date knowledge of hereditary α-tryptasemia as a frequent cause of baseline tryptase of 7 µg/L and higher, mastocytosis, other clonal myeloid disorders, cardiovascular or renal failure, and technical improvements resulting in continued lowering of the 95th percentile value are discussed.

Clues for improved management of perioperative immediate hypersensitivity and anaphylaxis include (i) sustained dissemination and implementation of updated guidelines; (ii) preoperative sample storage for deferred analysis; (ii) referral for thorough allergy investigation, screening for mast cell-related disorders and recommendations for future anesthetic procedures; (iii) sustained collaboration between anesthesiologists, immunologists, and allergists.

225 words

Keywords

algorithm; anaphylaxis; anesthesia; hypersensitivity; mast cell; perioperative; tryptase

Abbreviations

AFE, amniotic fluid embolism; FcεRI, high-affinity receptor for the Fc segment of IgE; FcγRI, high-affinity receptor for the Fc segment of IgG; FEIA, fluoro-enzyme immunoassay; GOF, gain-of-function; HaT, hereditary α-tryptasemia; Ig, immunoglobulin; mAb, monoclonal antibody; MRGPRX2, Mas-related G protein coupled receptor X2; NMBA, neuromuscular blocking agent; POA, perioperative anaphylaxis; POH, perioperative hypersensitivity; sAT, serum acute tryptase; sBT, serum baseline tryptase; THIQ, tetrahydroisoquinoline

1. Introduction: Definitions and epidemiology

Perioperative hypersensitivity (POH) is an immediate and potentially life threatening systemic reaction occurring during the perioperative period, defined as the time when the patient is under the care of an anesthesiologist[1] . The most severe POH reactions are referred to as perioperative anaphylaxis (POA). The lack of a universally accepted definition for POA or even anaphylaxis[2-3] led us to use the one proposed by the NAP6 (6th National Audit Project of the Royal College of Anaesthetists), i.e. grades III and IV of POH[1;4] **(Figure 1)**. Throughout this manuscript, unless otherwise stated, POH will denote any of its severity grades, including POA.

Although a rare event, POH is associated with significant morbidity and mortality and remains a management challenge for both anesthesiologists and allergists. In the clinical setting of the perioperative period, symptoms and signs compatible with POH may be difficult to distinguish from pharmacological effects of drugs, from effects of anesthetic or surgical procedures, from other medical emergencies (e.g., hypovolemic or cardiogenic shock), or from effects of inflammation.

The updated POH nomenclature (**Figure 1**) is based on that conventionally used for drug hypersensitivity and covers a wide variety of mechanisms[4-6]. The consistent use of this nomenclature will improve consistency across studies, and facilitate the analysis of POH incidence, management, and pathophysiology.

POH incidence is currently estimated as ranging from 1 reaction per 353 anesthetic procedures to 1 per 18,600[6-7]. POH reactions involving a presumed IgE mechanism have an estimated incidence of 1/5,000 to 1/13,000 anesthetic procedures, with data from France and the United Kingdom yielding a similar figure of 1/10,000 anesthetic procedures[8-9]. The reported mortality rate varies from 1.4% in Western Australia to 4.8% in Japan and has been estimated at 3.8% in the United Kingdom and 4.1% in France[9-11].

There are marked variations in POH incidence and causative agents from one country to another, due to differences in anesthetic agents, population sensitivities, and heterogeneity in the definition, allergist referral, and reporting of POH[4]. Such variations may be influenced by anesthetic practices, such as the preferred choice of neuromuscular blocking agents (NMBAs) or of antibiotics, which vary between countries[12]. Although still under investigation, exposure to pholcodine (3-o-morpholinoethylmorphine), an opioid cough suppressant available in only some countries, may predispose to NMBA reactions[13]. New culprits include disinfectants such as chlorhexidine and blue dyes, such as patent blue used in cancer surgery. Allergic reactions to other substances, such as hypnotics, opioids or local anesthetics, are quite rare[7] but some opioids can directly induce mast cells to degranulate and release histamine release[14].

Contribution of genetic factors and occupational exposures (e.g., quaternary ammonium in hairdressers and bakers) to the development of POH is also suspected[15-16].

Paired acute (sAT) and baseline (sBT) serum tryptase measurement provides a mechanistic approach in addition to the clinical signs. A transient elevation of sAT (optimally taken 30-120 min after onset of signs or symptoms; though depending on the magnitude of the peak sAT elevation, the level may still be elevated 4-6 h after onset) greater than [2 + (1.2x baseline tryptase level)] (baseline sample either retrieved from a sample drawn prior to the perioperative period or obtained at least 24 hours after all signs and symptoms have resolved) supports the involvement and activation of mast cells. Conversely, the lack of a transient elevation in serum tryptase during a hypotensive reaction supports a non-mast cell pathway being involved[6;17]. In all cases of POH suspicion, investigation is mandatory regardless of tryptase results (**Figure 2**). **(1st occurrence of references in Figure 2: 18-19)**

POH clinical presentation does not allow reliable discrimination of the underlying mechanism. Indeed, even in typical pictures with hypotension, tachycardia, wheezing and pruritic hives **(Figure 3a)**, tryptase measurements can be more precise for ascertaining mast cell activation, while allergy testing can best identify the trigger guiding future anesthetic choices **(Figure 3 b)**.**(1st occurrence of references in Figure 3: 20-22)**

2. Molecular mechanisms and pathophysiology of perioperative hypersensitivity and anaphylaxis

The molecular mechanisms and pathophysiology of POH have been reviewed in 2019[5]. Activation and degranulation of mast cells and basophils, occurring through various IgE:FcεRI-dependent and IgE:FcεRI-independent signaling pathways, play a pivotal role in POH.

Drugs, latex and other compounds used in the perioperative period can effectively cross-link IgE:FcεRI complexes on mast cells and basophils, initiating signal transduction and inducing the release of mediators[23]. Examples of IgE:FcεRI-dependent POH are reactions to β-lactam antibiotics, latex and chlorhexidine, as well as the majority of reactions to NMBAs. Limited evidence has suggested that the activation of mast cells and basophils can also be induced by antigen-specific IgG immune complexes which can aggregate FcγR2a and FcγRI on mast cells[24-28].

More recently, it was shown that mast cell activation by drugs from various classes such as NMBAs, opiates and quinolones can also result from binding to the Mas-related G protein coupled receptor-X2 (MRGPRX2)[29-31], particularly through a tetrahydroisoquinoline (THIQ) motif. However, current evidence for this novel mechanistic endotype predominantly comes from animal or *in vitro* studies and the clinical

relevance is uncertain. For example, the human mast cell line LAD-2 and primary cultured human mast cells could not be activated by rocuronium through MRGPRX2[32-33]. However, morphine, cisatracurium and vancomycin are ligands for MRGPRX2, and Red Man's Syndrome from this antibiotic seems to occur through this receptor on mast cells[32]. Studies have not been able to conclusively confirm the presence of MRGPRX2 on resting basophils[34-37], nor basophil activation by morphine[38] and the fluoroquinolone moxifloxacin[39].

MRGPRX2-dependent degranulation does not require prior sensitization to the culprit, occurs rapidly after exposure to ligands and is less likely to generate pro-inflammatory cytokines, chemokines and lipids mediators seen after IgE activation[40-41]. While all mast cells can be activated through the IgE:FcεRI pathways, only selected populations of mast cells have been shown to express MRGPRX2. Substance P, a natural ligand of MRGPRX2, induces significant histamine and tryptase release from human skin mast cells[42], which have high expression of this receptor[43]. Vancomycin-induced Red Man's Syndrome includes pruritus but not wheezing, consistent with the abundant presence of MRGPRX2 on skin but not lung-derived mast cells.

Direct activators of complement can generate vasoactive anaphylatoxins C5a and C3a, which bind to stereospecific G-protein-coupled receptor, C5a to C5aR (CD88) and C3a to C3aR, expressed by mast cells outside of the lung parenchyma and small intestinal mucosa[5]. Acute hypotensive reactions have been documented in dialysis patients when receiving over-sulfated chondroitin sulfate, a contaminant of heparin that activated the contact pathway, factor XII, leading to activation of plasma kallikrein and generation of bradykinin[44-45]. Various drugs, including penicillin G, when administered at suprapharmacologic concentrations, can activate the contact pathway in mice and rats and in human plasma[46], which does not occur at pharmacologic doses. Acute reactions to iodinated radiocontrast media have been reported to occur by complement activation[47], and more recently acute elevations in serum tryptase suggest that some cases involve mast cell activation[48].

3. Tryptase in the context of other anaphylaxis causes (Hymenoptera), mastocytosis, hereditary alpha tryptasemia, age and comorbidity-related variations

Tryptases genes are located on human chromosome 16 in two loci, *TPSB2* which encodes only β-tryptase and *TPSAB1*, encoding either α-tryptase or β tryptase. These tryptases are trypsin-like proteases primarily expressed by mast cells, and at a 200-fold lower level, on average, by basophils[49-50]. Production of α- and β- protryptase monomers takes place continuously in cultured mast cells, with a portion being constitutively secreted by unstimulated mast cells *in vitro*[51] and likely as well *in vivo*, accounting for nearly all of the tryptase detected in baseline samples of serum or plasma, a level that remains relatively constant for a given individual over time, dependent primarily on genetic factors[52]. Another portion of α- and β-protryptases are processed, in the presence of heparin at acidic pH, into mature forms that spontaneously form tetramers, α-tryptase homotetramers, β-tryptase homotetramers, and α/β-tryptase heterotetramers, that are stored in secretory granules in a complex with heparin proteoglycan, awaiting for mast cells to be activated to degranulate, whereupon the granule contents are externalized. The biological functions of tryptases are not well understood. α-tryptase lacks proteolytic activity, while β-tryptase and α/β-tryptase are proteolytically active. β-Tryptase can cleave fibrinogen destroying its ability to form fibrin when exposed to thrombin[53] and can directly generate C3a and C5a fragments from C3 and C5[54]. α/β-Tryptase, but not β-tryptase, directly activates protease-activated receptor-2 (PAR2) on human endothelial cells, increasing vasopermeability, and cleaves EMR2 (EGF-like module-containing mucin-like hormone receptor-like 2, CD312) on the surface of mast cells, making them susceptible to vibration-triggered degranulation[55-56], likely explaining some of the clinical features of hereditary alpha-tryptasemia. Unlike histamine, which rapidly diffuses after secretion, tryptase diffusion is limited by the macromolecular complexes in which it resides, delaying its appearance in the circulation compared to histamine. Thus, mature β tryptase will only be present in the bloodstream after mast cell activation and measurement of tryptase at this time is the sum of mature tryptases and the baseline protryptases.

Deficiency of α tryptase has not been associated with a clinical phenotype and is seen in individuals expressing only β tryptases at both TPSB2 and TPSAB1 locus. Its prevalence varies with one's ancestry, being highest in those with African ancestry (40%), then with European ancestry (23%), and lowest in Asian ancestry (10%)[57], suggesting that natural selection has occurred. Deficiency of active β-tryptase has not been reported. Current antibody tests used to measure tryptase in blood and biological fluids are based on common epitopes on α- and β- tryptases and cannot assess α-tryptase deficiency[58]. Median sBT level

measured with current Thermo Fisher ImmunoCAP assay in the general population is 3.6 µg/L. sBT levels in children are slightly lower than in adults, with a mean of about 3.4 µg/L and a tendency for boys to have higher levels than girls in some but not other studies[59-61].

Patients with systemic mastocytosis have a somatic gain-of-function (GOF) mutation of *c-KIT*, typically exhibit mast cell hyperplasia in the bone marrow and/or other organ systems, and have levels above 20 µg/L in about 75% of cases, with a small percentage having levels in the normal range[62]. Other conditions associated with elevated sBT levels include advanced renal failure and other clonal myelocytic disorders such as myelodysplastic syndrome associated with a somatic *JAK2* GOF mutation or hypereosinophilic syndrome associated with a somatic GOF mutation in *PDGFRA* or *PDGFRB*. Those with KIT GOF mutations can exhibit mast cell expansion and activation, likely due to ligand independent D816V mutated KIT activation, and thus are at increased risk for POH. Patients with coronary syndromes and acute changes in ST segment have been shown to present transiently elevated tryptase[63], with levels above 5 µg/L strongly predicting further major cardiovascular adverse events in the following 2 years[64]. sBT levels are increased in and predictive of chronic renal failure[65], although tryptase is not cleared by the kidneys into urine[66].
Hereditary α-tryptasemia (HaT), a recently described autosomal dominant disorder estimated to affect about 6% of those with a European ancestry, presents with extra copies of *TPSAB1*, but only when it encodes α-tryptase, and elevated sBT levels (>7 µg/L)[56;67-71]. Although most affected families have only one extra gene copy, up to four extra gene copies have been reported[72]. The more extra copies of *TPSAB1* within a family, the higher is the sBT level, the higher is the portion of active mast cell tryptase accounted for by α/β-tryptase heterotetramers, and the greater is the symptom burden – though some individuals with this genetic trait have no symptoms. HaT, mastocytosis, and other clonal mast cell disorders are distinct conditions that can occur independently or in association. Current knowledge indicates that HaT patients are at higher risk for more severe spontaneous or Hymenoptera sting-triggered anaphylaxis, while systemic mastocytosis patients exhibit higher incidence and severity for such events, and there is a cumulative effect in people diagnosed with both mastocytosis and HaT, who experience the highest prevalence of such reactions[56;71]. Thus, HaT likely explains the early observation that the risk for Hymenoptera sting-induced anaphylaxis markedly increased in people with sBT levels above 5 µg/L[72].
Although whether HaT confers an increased risk for POH has not yet been studied, a reasonable hypothesis to consider is whether the severity of POH may be higher than in an unaffected control group. This hypothesis is supported by the observation of more frequent sBT greater than 5 µg/L among patients having experienced more severe POH[74].

4. Review of evidence (including pitfalls) and current official recommendations for acute and baseline tryptase level measurement as a tool for perioperative hypersensitivity and anaphylaxis

The measurement of serum tryptase is performed with a commercially available immunoassay that measures the mature and pro forms of α- and β- tryptases, referred to as "total tryptase" (ImmunoCAP Tryptase, Thermo Fisher Scientific, Uppsala, Sweden). A timeline of conceptual and methodological progress in the field of tryptase is presented in **Figure 4**. (**1st occurrence of references in Figure 4: 75-87**)

Briefly, tryptase measurement for anaphylaxis was proposed in 1987, based on the first tryptase assay[77;79], which only later was recognized as detecting mature forms of α- and β- tryptases, but not their pro forms. Circulating mature tryptase levels higher than the detection threshold were found in acute samples from anaphylaxis and baseline samples from mastocytosis patients, but not in samples from healthy donors. This led to development of a radioimmune tryptase assay[78] that also turned out to measure only the mature forms of the protein. In 1994, using new anti-tryptase monoclonal antibodies (mAbs), a new immunoassay was developed that could detect tryptase levels at baseline in most individuals[58], because as later learned it detected pro- as well as mature forms of α- and β- tryptases. The total tryptase assay quantitated circulating tryptase not only in anaphylaxis and mastocytosis, but also in healthy controls, with significant interindividual variations[58]. Minor modifications of the 1994 total tryptase assay resulted in the commercial fluoro-enzyme immunoassay (FEIA) test released in 1995 (Pharmacia & Upjohn, then Phadia and now Thermo Fisher Scientific, Uppsala, Sweden), using the B12 anti-tryptase mAb for capture and the G4 anti-tryptase mAb for detection. Modifications since then include the addition of an agent to suppress heterophilic antibodies that could produce false elevations, replacement of purified lung-derived tryptase used as standards with recombinant human β-protryptase, and converting the G4 anti-tryptase IgG mAb to its $F(ab')_2$ form. Virtually all clinical tryptase determinations worldwide have been performed with this commercial assay for the last 25 years. Thus, using a total tryptase assay improved the precision for diagnosing mast cell-mediated hypersensitivity, including POH, requiring the serum acute tryptase (sAT) level (collected 30-120 min after clinical onset) be higher than [2 + (1.2xsBT)]. The sBT should be collected either before the reaction or at least 24 hours after all signs and symptoms have resolved[58;74;84-89]. Using this algorithm is more specific and sensitive than using sAT alone.

For insect sting-triggered systemic anaphylaxis, taking the onset of symptoms as the reference time point, tryptase elevation is detectable in peripheral blood after a latency of 15-30 minutes; a maximum is reached

at approximately 1 hour, followed by a decline to baseline levels of about 50% every 2 hours[58;80]. sBT levels have been shown to be very reproducible, except for a negligible dilutional effect, in the perioperative setting in the absence of hypersensitivity[83;89]. To date, serum tryptase is the principal mast cell biomarker available for in vitro diagnostics, and its interpretation is straightforward, with a consensus algorithm proposed in 2012[84], validated in several studies during the last decade, and which is now recommended in guidelines of several organizations for diagnosing anaphylaxis in general and in the perioperative setting[6;85;87]. This algorithm allows calculating an individual cut-off for each patient, based on sAT and sBT values: sAT exceeding [2 + (1.2 x sBT)] µg/L supports mast cell degranulation, even in cases when sAT remains in the normal reference range[74;84-87;89]. However, paired sAT and sBT determination in the highly complex setting of suspected POH is not optimally implemented in current practice in many hospitals.

In the perioperative setting, commonly occurring events such as the sudden onset of hypotension, tachycardia and/or bronchospasm, may be interpreted as hypersensitivity, but in fact may be caused by other factors not related to hypersensitivity. In contrast, analysis of tryptase measurements and allergy investigations, respectively, revealed mast cell activation and a likely allergic trigger for some perioperative reactions when the initial clinical evaluation did not favor such a diagnosis POH[90]. In addition to the allergist discussing the reaction and potential differential diagnoses with an anesthesiologist, biomarkers, notably serum tryptase, are recommended to be included in the determination of mast cell involvement and severity grading[6]. This is an important step to acknowledge, because anaphylaxis has always been considered as a clinical diagnosis, enjoying a strong transgenerational consensus despite the fact that the clinical presentation alone is too often misunderstood or misdiagnosed. In fact, there is a double need of immediate recognition of possible anaphylaxis prompting appropriate epinephrine treatment, followed by immediate collection of elements substantiating the hypersensitivity mechanism, including serum tryptase sampling. This has been addressed in recent guidelines[6;91-92]. The updated references rely on correctly paired sAT with sBT sampling for the diagnosis of POH, providing greater precision for determining whether mast cell activation indeed occurred. The timing of this paired sampling is clearly defined in international consensus papers, with the optimal window for sAT sampling between 30 minutes and 2 hours after the initiation of symptoms, while sBT should be measured in a sample collected before the event (pre-operative) or at least 24 hours after all signs and symptoms have resolved[6;18;84-87;93]. Physicians must be aware that in some patients, especially those with very high sAT levels, tryptase measured 24 h after clinical onset might still be elevated, though to remain elevated 24 h after all signs and symptoms have resolved is rare. In such a case, a control sample at a later time might be warranted. The gradual post-reaction decrease of serum tryptase levels (with a half-life around 2 h after peak level is reached)

explains why sampling at later times, three or even four hours post-reaction was accepted for practical reasons for outpatients in earlier guidelines[94-96]. Although delayed sAT sampling may still be the only option in patients experiencing hypersensitivity reactions outside the hospital, proper timing for sAT during POH is achievable, and this is acknowledged in current guidelines because it provides better diagnostic sensitivity for mast cell degranulation,

In recent years, sAT and sBT sampling for suspicion of POH has become more common. A properly timed sAT is available in many centers where POH awareness and collaboration between anesthesiologists, allergists, and immunologists are well established. Recent figures may be as high as 86% in UK patients[97], 99% in French patients from Marseille[74], and 80% in Danish patients[91], showing better guideline implementation than in earlier reports from the same countries, e.g. 41% in Flanders[98], or 67% in France[17].

On the other hand, proper sBT determination is still the poor cousin, overlooked in as many as 15-25% of patients[74;97]. The most common reasons appear to be the erroneous assumptions that a sAT value exceeding the manufacturer's reference level of the 95th upper percentile of apparently healthy donor groups, or other cut-off levels usually established by personal experience, provides a sufficiently sensitive marker of perioperative mast cell degranulation, while a sAT value lower than such a reference does not exclude the diagnosis of POH, as an sAT of 4 can be clinically significant if the sBT is 1. However, an sAT of 3 or lower would exclude mast cell activation by the tryptase algorithm. In fact, neither a universal reference level, nor an isolated sAT result are reliable criteria.

Technical improvements of the total tryptase assay resulted in continued lowering of the manufacturer's 95th upper percentile value: currently 11 µg/L, previously 11.4 µg/L and 14 µg/L (**Figure 4**).

sAT cannot be interpreted without the sBT level of the patient. Assuming on statistical grounds that a given patient's sBT is normal and therefore omitting to measure it conveys risks for the patient. Elevated sBT is not an uncommon finding, e.g., 10% of patients with POA displayed sBT greater than 15.4 µg/L in the UK NAP 6[97]. HaT, mastocytosis, other clonal myeloid disorders or renal failure are associated with sBT levels above the normal range. Current knowledge places HaT as the most frequent cause of an sBT of 7 µg/L and higher in about 6% of those with a European ancestry, less in those with an Asian or African ancestry[69]. The demonstration of similarly elevated sAT and sBT levels may not only prevent an incorrect diagnosis of MC activation, but also can help identify an alternative or underlying diagnosis. Moreover, missing a permanent mast cell-related condition, including mastocytosis and/or HAT, often discovered as an elevated sBT[56;99-100], means failing to give that individual patient the best possible diagnostic information and care.

Conversely, sAT levels do not reach 11.5 µg/L in all patients experiencing POH reactions. Early evidence[58] consolidated during the past decade showed that elevation of sAT is linked to clinical severity, with hypotension being the best clinical correlate, explaining the 10 to 60% prevalence of sAT figures below 11.5 µg/L reported in the literature[74;97-98;101]. sBT measurement is irreplaceable for the interpretation of such apparently "normal" sAT values.

Another indirect cause of insufficient sBT determination is the universally low rate of referral to an allergist for patients having experienced a suspected POH[74;98]. Indeed, proper referral for an allergy work-up in the weeks or months following a suspected hypersensitivity reaction is advised[6], and should be accompanied by a list of all perioperative medications, topical agents and materials containing latex, animal products or other potential allergens. Allergy work-up provides an opportunity to check sBT in case this has not been done under the anesthesiologists' supervision, as recommended in adults and children alike[6;102].

Finally, the availability of *in vitro* diagnostics and the expense of two tryptase determinations are sometimes cited as limiting factors for the proper implementation of the recommended two-tryptase scheme. We believe that state-of-the-art recommendations must be supported and used as an incentive for improving local practice. Importantly, serum samples can be stored and assayed later. This is true for sAT sampling, but also for sBT as a provisional pre-operative serum sampling which might be used for sBT measurement in case of subsequent perioperative reaction. Currently, testing for sBT in all patients referred for an operative procedure is not recommended. However, if serum is drawn for pre-operative blood tests, a portion can be retrieved to measure the sBT level in case a POH occurs. Referral to an allergist is strongly recommended, and allows for thorough patient screening for mast cell-related disorders. A collaboration of anesthesiologists with allergists is the best solution for continued improvement of POH management.

A noteworthy shortcoming for using the tryptase algorithm is that a genuine IgE-mediated hypersensitivity reaction may not be revealed if a systemic reaction is of low severity, particularly in the absence of hypotension, or if the reaction occurs at a local site. Indeed, the rise in tryptase levels during hypersensitivity reactions correlates primarily with the magnitude of hypotension. In the absence of hypotension, isolated cutaneous, gastrointestinal or respiratory manifestations, even though locally severe and associated with local mast cell activation, may not raise tryptase levels in the circulation. Hypotension might reflect activation of mast cells in blood vessel walls, from where tryptase might diffuse more readily into the circulation than from other sites. Another explanation might be the reaction is due to other cells than mast cells, or to newly generated vasoactive mediators being secreted rather than to

degranulation-dependent release of stored mediators[25;103]. Basophil contributions to tryptase levels is limited, because they contain much less tryptase than mast cells[49-50;104]. Finally, activation of non-mast cell pathways may mimic signs and symptoms of anaphylaxis, as reported when over-sulfated chondroitin sulfate was inadvertently administered to patients and acutely activated the contact pathway, resulting in overproduction of vasoactive bradykinin[44;105], or when an older type of dialysis membrane acutely activated the complement pathway, which generates vasoactive C3a and C5a anaphylatoxins[106].

The so-called dilution effect, stating that massive infusion of liquids at the onset of perioperative deterioration, might lead to an underestimation of sAT, has been experimentally refuted, as 1-2 L of normal saline result in a negligible dilutional effect, and minimal variation of tryptase levels are observed outside POH[83;89].

Tryptase is a reliable analyte in particular situations, such as pediatric POH and POH during pregnancy. Although pediatric POH is a rare event, accounting for less than 10% of total POH events and 1 in 37,000 pediatric anesthetic procedures[98;107-108], it may be severe[98;107;109]. The largest series of pediatric POH reported 266 cases[8]. The top three culprits are latex, NMBAs, and antibiotics[8;110]. In 1177 children treated postoperatively for pain with metamizole the probability of serious allergic reactions and anaphylaxis was 0.3%[111]. Tryptase determination performs similarly to adults[102;109;112].

POH during an obstetrical procedure is an even more rare event, with a reported incidence of 3 in 100,000 deliveries[113], and an estimated incidence for the whole duration of pregnancy of 1.5 in 100,000 in Europe[114]. The management of POH suspicion in pregnancy, including sAT and sBT sampling, is the same as in general population[114-115]. The rate of tryptase sampling in POH during pregnancy was recently reported to be 86% in the UK, but only 54% in continental European countries[114]. Of note, tryptase assessment is unaffected by pregnancy-related high levels of diamine-oxidase, as opposed to histamine, which is degraded by this enzyme, leading to false negative results[116]. Amniotic fluid embolism (AFE) may occur during obstetrical anesthesia and present as a clinical differential diagnosis of POH[117-118].

Post-mortem determination of tryptase has been proposed for the diagnosis of fatal anaphylaxis[119], including fatal POH, in cases when sAT sampling could not be performed, e.g., the death occurred perioperatively. The collection site for *post-mortem* tryptase may influence the results, and it is therefore advised that *post-mortem* sampling for tryptase determination should be done from the femoral vein[120]. Similarly, sBT levels cannot be obtained after the putative POH event unless the death occurs more than 24 hours after resolution of this event. If serum or plasma is available from before the event and was appropriately stored, then it could be used as a baseline. Overall, paired sAT and sBT are seldom available

in this context, explaining why the general sAT > [2+(1.2 x sBT)] equation is often not applicable, and a consensus cut-off value for sAT is lacking[121-122]. The higher the value, the higher the probability that mast cell activation was involved with the putative POH event. Perioperative tryptase levels are not affected by resuscitation procedures and are not elevated in patients who die during anesthesia from non-allergic causes[123]. Outside POH, raised *post-mortem* tryptase levels have been reported in isolated cases of *pre-mortem* trauma, myocardial infarction, asphyxia, and pulmonary damage[121;124].

5. Combined clinical + dual tryptase score for perioperative hypersensitivity and anaphylaxis

We recommend sAT sampling at 30 min to 2 h after the onset of symptoms, and sBT from either a blood sample collected before the event or one collected at least 24h after complete resolution of symptoms and signs of anaphylaxis. In some patients, tryptase measured at 24 h after onset might still be elevated, and a control sample at a later time might be warranted, well after all signs and symptoms have resolved. Referral to an allergist must be part of the diagnostic procedure for operative centers. The complete recommended algorithm for tryptase sampling during POH is presented in **Figure 5**.

6. Unmet needs, research perspectives and concluding remarks

Pediatric POH is a rare event, and published data are scarce. Larger series are needed in order to better understand and manage POH in this population and using the above algorithm with measurements of tryptase during the acute event and obtaining a baseline tryptase is recommended.

Once POH has occurred, referral to allergist is mandatory regardless of tryptase elevation, to improve patient safety and provide recommendations for future anesthetic procedures. If epinephrine is used an automatic prompt for tryptase determination should be implemented in all electronic health care systems and integrated in all anaphylaxis/hypersensitivity algorithms aided by artificial intelligence which would aid in the recognition of the symptoms of hypersensitivity and anaphylaxis. Rapid automated tryptase determination is technically possible and would be of great help at bedside. Adding rapid sAT determination in the operation room to blood sampling for laboratory sAT and sBT determination would contribute to better recognition and management of POH at early stages. Beyond tryptase, transdermal and real time measurements of mediators and physical signs are needed for a better assessment, diagnosis, and treatment of POH.

Acknowledgments

Bjarne Kristensen, Nordic Area Manager, ImmunoDiagnostics, Thermo Fisher Scientific, Allerød, Denmark, and Niclas U. Rydell, Senior Scientist, ImmunoDiagnostics, Thermo Fisher Scientific, Phadia AB, Uppsala, Sweden for generously providing information related to the development of commercial tryptase assays.

References

1. Cook TM, Harper NJN, Farmer L, Garcez T, Floss K, Marinho S, et al. Anaesthesia, surgery, and life-threatening allergic reactions: protocol and methods of the 6th National Audit Project (NAP6) of the Royal College of Anaesthetists. Br J Anaesth 2018;121:124–33.
2. Sampson HA, Muñoz-Furlong A, Campbell RL, Adkinson NF Jr, Bock SA, Branum A, 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.
3. de Silva D, Singh C, Muraro A, Worm M, Alviani C, Cardona V, et al; European Academy of Allergy and Clinical Immunology Food Allergy and Anaphylaxis Guidelines Group. Diagnosing, managing and preventing anaphylaxis: Systematic review. Allergy 2020 Sep 2. doi: 10.1111/all.14580. Online ahead of print.
4. Sabato V, Platt P, Garcez T, Cooke P. Suspected perioperative allergic reactions: nomenclature and terminology. Br J Anaesth 2019;123:e13–5.
5. Ebo DG, Clarke RC, Mertes P-M, Platt PR, Sabato V, Sadleir PHM. Molecular mechanisms and pathophysiology of perioperative hypersensitivity and anaphylaxis: a narrative review. Br J Anaesth 2019;123:e38–49.
6. Garvey LH, Ebo DG, Mertes P-M, Dewachter P, Garcez T, Kopac P, et al. An EAACI position paper on the investigation of perioperative immediate hypersensitivity reactions. Allergy 2019;74:1872–84.
7. Mertes PM, Ebo DG, Garcez T, Rose M, Sabato V, Takazawa T, et al. Comparative epidemiology of suspected perioperative hypersensitivity reactions. Br J Anaesth 2019;123:e16–28.
8. Mertes PM, Alla F, Tréchot P, Auroy Y, Jougla E, Groupe d'Etudes des Réactions Anaphylactoïdes Peranesthésiques. Anaphylaxis during anesthesia in France: an 8-year national survey. J Allergy Clin Immunol 2011;128:366–73.
9. Harper NJN, Cook TM, Garcez T, Farmer L, Floss K, Marinho S, et al. Anaesthesia, surgery, and life-threatening allergic reactions: epidemiology and clinical features of perioperative anaphylaxis in the 6th National Audit Project (NAP6). Br J Anaesth 2018;121:159–71.
10. Gibbs NM, Sadleir PH, Clarke RC, Platt PR. Survival from perioperative anaphylaxis in Western Australia 2000-2009. Br J Anaesth 2013;111:589–93.

11. Reitter M, Petitpain N, Latarche C, Cottin J, Massy N, Demoly P, et al. Fatal anaphylaxis with neuromuscular blocking agents: a risk factor and management analysis. Allergy 2014;69:954–9.
12. Garvey LH. Old, New and Hidden Causes of Perioperative Hypersensitivity. Curr Pharm Des 2016;22:6814-24. doi: 10.2174/1381612822666161004125143.
13. Johansson SGO, Florvaag E, Oman H, Poulsen LK, Mertes PM, Harper NJN, et al. National pholcodine consumption and prevalence of IgE-sensitization: a multicentre study. Allergy 2010;65:498–502.
14. Lepelley M, Khouri C, Pralong P, Rossignol J, Greco C, Bouillet L, et al. Which opioids in case of mast cell activation disorders? J Allergy Clin Immunol Pract. 2019 Apr;7(4):1317-1318. doi: 10.1016/j.jaip.2018.08.011.
15. Guéant JL, Guéant-Rodriguez RM, Gastin IA, Cornejo-García JA, Viola M, Barbaud A, et al. Pharmacogenetic determinants of immediate and delayed reactions of drug hypersensitivity. Curr Pharm Des 2008;14:2770–7.
16. Dong S, Acouetey DS, Guéant-Rodriguez R-M, Zmirou-Navier D, Rémen T, Blanca M, et al. Prevalence of IgE against neuromuscular blocking agents in hairdressers and bakers. Clin Exp Allergy 2013;43:1256–62.
17. Tacquard C, Collange O, Gomis P, Malinovsky J-M, Petitpain N, Demoly P, et al. Anaesthetic hypersensitivity reactions in France between 2011 and 2012: the 10th GERAP epidemiologic survey. Acta Anaesthesiol Scand 2017;61:290–9.
18. Garvey LH, Dewachter P, Hepner DL, Mertes PM, Voltolini S, Clarke R, et al. Management of suspected immediate perioperative allergic reactions: an international overview and consensus recommendations. Br J Anaesth 2019;123:e50–64.
19. Mullins RJ, James H, Platts-Mills TA, Commins S. Relationship between red meat allergy and sensitization to gelatin and galactose-α-1,3-galactose. J Allergy Clin Immunol 2012;129:1334-42.e1. doi: 10.1016/j.jaci.2012.02.038.
20. Dewachter P, Kopac P, Laguna JJ, Mertes PM, Sabato V, Volcheck GW, et al. Anaesthetic management of patients with pre-existing allergic conditions: a narrative review. Br J Anaesth 2019;123:e65-e81. doi: 10.1016/j.bja.2019.01.020.
21. Bonadonna P, Pagani M, Aberer W, Bilò MB, Brockow K, Oude Elberink H, et al. Drug hypersensitivity in clonal mast cell disorders: ENDA/EAACI position paper. Allergy 2015;70:755-63. doi: 10.1111/all.12617.

22. Castells M, Butterfield J. Mast Cell Activation Syndrome and Mastocytosis: Initial Treatment Options and Long-Term Management. J Allergy Clin Immunol Pract 2019;7:1097-106. doi: 10.1016/j.jaip.2019.02.002.
23. Knol EF. Requirements for effective IgE cross-linking on mast cells and basophils. Mol Nutr Food Res 2006;50:620–4.
24. Finkelman FD, Khodoun MV, Strait R. Human IgE-independent systemic anaphylaxis. J Allergy Clin Immunol 2016;137:1674–80.
25. Reber LL, Hernandez JD, Galli SJ. The pathophysiology of anaphylaxis. J Allergy Clin Immunol 2017;140:335–48.
26. Jönsson F, de Chaisemartin L, Granger V, Gouel-Chéron A, Gillis CM, Zhu Q, et al. An IgG-induced neutrophil activation pathway contributes to human drug-induced anaphylaxis. Sci Transl Med 2019;11(500).
27. Renck H, Ljungström KG, Rosberg B, Dhunér KG, Dahl S. Prevention of dextran-induced anaphylactic reactions by hapten inhibition. II. A comparison of the effects of 20 ml dextran 1, 15%, administered either admixed to or before dextran 70 or dextran 40. Acta Chir Scand 1983;149:349–53.
28. Kraft D, Hedin H, Richter W, Scheiner O, Rumpold H, Devey ME. Immunoglobulin class and subclass distribution of dextran-reactive antibodies in human reactors and non reactors to clinical dextran. Allergy 1982;37:481–9.
29. McNeil BD, Pundir P, Meeker S, Han L, Undem BJ, Kulka M, et al. Identification of a mast-cell-specific receptor crucial for pseudo-allergic drug reactions. Nature 2015;519:237–41.
30. Subramanian H, Gupta K, Ali H. Roles of Mas-related G protein-coupled receptor X2 on mast cell-mediated host defense, pseudoallergic drug reactions, and chronic inflammatory diseases. J Allergy Clin Immunol 2016; 138:700-10. doi: 10.1016/j.jaci.2016.04.051.
31. Van Gasse AL, Elst J, Bridts CH, Mertens C, Faber M, Hagendorens MM, et al. Rocuronium Hypersensitivity: Does Off-Target Occupation of the MRGPRX2 Receptor Play a Role? J Allergy Clin Immunol Pract. 2019 Mar;7(3):998-1003. doi: 10.1016/j.jaip.2018.09.034.
32. Navinés-Ferrer A, Serrano-Candelas E, Lafuente A, Muñoz-Cano R, Martín M, Gastaminza G. MRGPRX2-mediated mast cell response to drugs used in perioperative procedures and anaesthesia. Sci Rep 2018;8:11628.

33. Elst J, Sabato V, Faber MA, Bridts CH, Mertens C, Van Houdt M, et al. MRGPRX2 and Immediate Drug Hypersensitivity: Insights from Cultured Human Mast Cells. J Investig Allergol Clin Immunol 2020 Jul 30; doi: 10.18176/jiaci.0557. Online ahead of print.
34. Wedi B, Gehring M, Kapp A. The pseudoallergen receptor MRGPRX2 on peripheral blood basophils and eosinophils: expression and function. Allergy 2020;75:2229-42. doi: 10.1111/all.14213.
35. Sabato V, Elst J, Van Houdt M, Bridts C, Mertens C, Ebo DG. Surface expression of MRGPRX2 on resting basophils: An area of controversy. Allergy. 2020;75:2421–2.
36. Sabato V, Gasse AV, Cop N, Claesen K, Decuyper II, Faber MA, et al. The Mas-Related G Protein-Coupled Receptor MRGPRX2 Is Expressed on Human Basophils and up-Regulated upon Activation. J Allergy Clin Immunol 2017;139:AB168.
37. Elst J, Sabato V, Hagendorens MM, van Houdt M, Faber MA, Bridts CH, et al. Measurement and Functional Analysis of the Mas-Related G Protein-Coupled Receptor MRGPRX2 on Human Mast Cells and Basophils. Methods Mol Biol 2020;2163:219–26.
38. Van Gasse AL, Hagendorens MM, Sabato V, Bridts CH, De Clerck LS, Ebo DG. IgE to Poppy Seed and Morphine Are Not Useful Tools to Diagnose Opiate Allergy. J Allergy Clin Immunol Pract 2015;3:396–9.
39. Van Gasse AL, Sabato V, Uyttebroek AP, Elst J, Faber MA, Hagendorens MM, et al. Immediate moxifloxacin hypersensitivity: Is there more than currently meets the eye? Allergy 2017;72:2039–43.
40. Gaudenzio N, Sibilano R, Marichal T, Starkl P, Reber LL, Cenac N, et al. Different activation signals induce distinct mast cell degranulation strategies. J Clin Invest 2016;126:3981–98.
41. Karhausen J, Abraham SN. How mast cells make decisions. J Clin Invest 2016;126:3735–8.
42. Varricchi G, Pecoraro A, Loffredo S, Poto R, Rivellese F, Genovese A, et al. Heterogeneity of Human Mast Cells With Respect to MRGPRX2 Receptor Expression and Function. Front Cell Neurosci 2019;13:299.
43. Tatemoto K, Nozaki Y, Tsuda R, Konno S, Tomura K, Furuno M, et al. Immunoglobulin E-independent activation of mast cell is mediated by Mrg receptors. Biochem Biophys Res Commun 2006;349:1322–8.
44. Kishimoto TK, Viswanathan K, Ganguly T, Elankumaran S, Smith S, Pelzer K, et al. Contaminated heparin associated with adverse clinical events and activation of the contact system. N Engl J Med 2008;358:2457–67.

45. Zhou Z-H, Chen T, Arora K, Hyams K, Kozlowski S. Complement C1 esterase inhibitor levels linked to infections and contaminated heparin-associated adverse events. PloS One 2012;7:e34978.
46. Gao Y, Han Y, Zhang X, Fei Q, Qi R, Hou R, et al. Penicillin causes non-allergic anaphylaxis by activating the contact system. Sci Rep 2020;10:14160.
47. Arroyave CM, Tan EM. Mechanism of complement activation by radiographic contrast media. Clin Exp Immunol 1977;29:89–94.
48. Brockow K, Sanchez-Borges M. Hypersensitivity to contrast media and dyes. Immunol Allergy Clin North Am 2014;34: 547-564.
49. Castells MC, Irani AM, Schwartz LB. Evaluation of human peripheral blood leukocytes for mast cell tryptase. J Immunol 1987;138:2184–9.
50. Jogie-Brahim S, Min H-K, Fukuoka Y, Xia H-Z, Schwartz LB. Expression of alpha-tryptase and beta-tryptase by human basophils. J Allergy Clin Immunol 2004;113:1086–92.
51. Schwartz LB, Min HK, Ren S, Xia HZ, Hu J, Zhao W, et al. Tryptase precursors are preferentially and spontaneously released, whereas mature tryptase is retained by HMC-1 cells, mono-mac-6 cells, and human skin-derived mast cells. J Immunol 2003;170: 5667-73.
52. Sverrild A, van der Sluis S, Kyvik KO, Garvey LH, Porsbjerg C, Backer V, et al. Genetic factors account for most of the variation in serum tryptase-a twin study. Ann Allergy Asthma Immunol 2013;111:286-9.
53. Prieto-García A, Pérez-David E, Devesa C, Tornero P, Schwartz LB, Pascual C, et al. Fatal anaphylaxis caused by gadolinium due to beta-tryptase-induced hemorragic diathesis. J Allergy Clin Immunol Pract 2017;5:1433–4.
54. Fukuoka Y, Xia H-Z, Sanchez-Muñoz LB, Dellinger AL, Escribano L, Schwartz LB. Generation of anaphylatoxins by human beta-tryptase from C3, C4, and C5. J Immunol 2008;180:6307–16.
55. Le QT, Lyons JJ, Naranjo AN, Olivera A, Lazarus RA, Metcalfe DD, et al. Impact of naturally forming human alpha/beta-tryptase heterotetramers in the pathogenesis of hereditary alpha-tryptasemia. J Exp Med 2019;216:2348-61.
56. Lyons JJ, Chovanec J, O'Connell MP, Liu Y, Šelb J, Zanotti R, et al. Heritable risk for severe anaphylaxis associated with increased α-tryptase-encoding germline copy number at TPSAB1. J Allergy Clin Immunol. 2020 Jul 24; S0091-6749(20)31029-0. doi: 10.1016/j.jaci.2020.06.035.
57. Trivedi NN, Tamraz B, Chu C, Kwok PY, Caughey GH. Human subjects are protected from mast cell tryptase deficiency despite frequent inheritance of loss-of-function mutations. J Allergy Clin Immunol 2009;124:1099-105.e1-4.

589 58. Schwartz LB, Bradford TR, Rouse C, Irani AM, Rasp G, Van der Zwan JK, et al. Development of a
590 new, more sensitive immunoassay for human tryptase: use in systemic anaphylaxis. J Clin Immunol
591 1994;14:190–204.
592 59. Komarow HD, Hu Z, Brittain E, Uzzaman A, Gaskins D, Metcalfe DD. Serum tryptase levels in atopic
593 and nonatopic children. J Allergy Clin Immunol 2009;124:845–8.
594 60. Belhocine W, Ibrahim Z, Grandné V, Buffat C, Robert P, Gras D, et al. Total serum tryptase levels
595 are higher in young infants. Pediatr Allergy Immunol 2011;22:600–7.
596 61. Sahiner UM, Yavuz ST, Buyuktiryaki B, Cavkaytar O, Arik Yilmaz E, Tuncer A, et al. Serum basal
597 tryptase levels in healthy children: correlation between age and gender. Allergy Asthma Proc
598 2014;35:404–8.
599 62. Valent P, Akin C, Metcalfe DD. Mastocytosis: 2016 updated WHO classification and novel emerging
600 treatment concepts. Blood 2017;129:1420-7.
601 63. Lewicki L, Siebert J, Marek-Trzonkowska N, Masiewicz E, Kolinski T, Reiwer-Gostomska M, et al.
602 Elevated Serum Tryptase and Endothelin in Patients with ST Segment Elevation Myocardial
603 Infarction: Preliminary Report. *Mediators Inflamm* 2015;2015:395173.
604 64. Pastorello EA, Farioli L, Losappio LM, Morici N, Di Biase M, Nichelatti M, et al. Serum tryptase
605 detected during acute coronary syndrome is significantly related to the development of major
606 adverse cardiovascular events after 2 years. Clin Mol Allergy 2015;13:14.
607 65. Jesky MD, Stringer SJ, Fenton A, Ng KP, Yadav P, Ndumbo M, et al. Serum tryptase concentration
608 and progression to end-stage renal disease. Eur J Clin Invest 2016;46:460-74.
609 66. Simon MR, Jan M, Yee J, Nori US, Hu J, Akin C, et al. Tryptase is not cleared by the kidneys into the
610 urine. Int Arch Allergy Immunol. 2010;152(1):28-31. doi: 10.1159/000260080.
611 67. Sabato V, Van De Vijver E, Hagendorens M, Vrelust I, Reyniers E, Fransen E, et al. Familial
612 hypertryptasemia with associated mast cell activation syndrome. J Allergy Clin Immunol
613 2014;134:1448-50.e3.
614 68. Lyons JJ, Yu X, Hughes JD, Le QT, Jamil A, Bai Y, et al. Elevated basal serum tryptase identifies a
615 multisystem disorder associated with increased TPSAB1 copy number. Nat Genet 2016;48:1564–
616 9.
617 69. Robey RC, Wilcock A, Bonin H, Beaman G, Myers B, Grattan C, et al. Hereditary Alpha-Tryptasemia:
618 UK Prevalence and Variability in Disease Expression. J Allergy Clin Immunol Pract 2020;8:3549–56.

70. Lyons JJ, Sun G, Stone KD, Nelson C, Wisch L, O'Brien M, et al. Mendelian inheritance of elevated serum tryptase associated with atopy and connective tissue abnormalities. J Allergy Clin Immunol 2014;133:1471–4.
71. Greiner G, Sprinzl B, Górska A, Ratzinger F, Gurbisz M, Witzeneder N, et al. Hereditary alpha tryptasemia is a valid genetic biomarker for severe mediator-related symptoms in mastocytosis. Blood 2020 Aug 10; blood.2020006157. doi: 10.1182/blood.2020006157. PMID: 32777817
72. Sabato V, Chovanec J, Faber M, Milner JD, Ebo D, Lyons JJ. First Identification of an Inherited TPSAB1 Quintuplication in a Patient with Clonal Mast Cell Disease. J Clin Immunol. 2018 May;38(4):457-459. doi: 10.1007/s10875-018-0506-y.
73. Ruëff F, Przybilla B, Biló MB, Müller U, Scheipl F, Aberer W, et al. Predictors of severe systemic anaphylactic reactions in patients with Hymenoptera venom allergy: importance of baseline serum tryptase-a study of the European Academy of Allergology and Clinical Immunology Interest Group on Insect Venom Hypersensitivity. J Allergy Clin Immunol 2009;124:1047–54.
74. 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.
75. Schwartz LB, Lewis RA, Austen KF. Tryptase from human pulmonary mast cells. Purification and characterization. J Biol Chem 1981;256:11939-43.
76. Schwartz LB, Lewis RA, Seldin D, Austen KF. Acid hydrolases and tryptase from secretory granules of dispersed human lung mast cells. J Immunol 1981;126: 1290-4.
77. Wenzel S, Irani AM, Sanders JM, Bradford TR, Schwartz LB. Immunoassay of tryptase from human mast cells. J Immunol Methods 1986;86:139–42.
78. Enander I, Matsson P, Nystrand J, Andersson AS, Eklund E, Bradford TR, et al. A new radioimmunoassay for human mast cell tryptase using monoclonal antibodies. J Immunol Methods 1991;138:39–46.
79. Schwartz LB, Metcalfe DD, Miller JS, Earl H, Sullivan T. Tryptase levels as an indicator of mast-cell activation in systemic anaphylaxis and mastocytosis. N Engl J Med 1987;316:1622–6.
80. 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.

81. Lin RY, Schwartz LB, Curry A, Pesola GR, Knight RJ, Lee HS, et al. Histamine and tryptase levels in patients with acute allergic reactions: An emergency department-based study. J Allergy Clin Immunol 2000;106(1 Pt 1):65-71.
82. Valent P, Horny HP, Escribano L, Longley BJ, Li CY, Schwartz LB, et al. Diagnostic criteria and classification of mastocytosis: a consensus proposal. Leuk Res 2001;25:603-25.
83. Garvey LH, Bech B, Mosbech H, Krøigaard M, Belhage B, Husum B, et al. Effect of general anesthesia and orthopedic surgery on serum tryptase. Anesthesiology 2010;112:1184–9.
84. 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.
85. Valent P, Akin C, Bonadonna P, Hartmann K, Brockow K, Niedoszytko M, et al. Proposed Diagnostic Algorithm for Patients with Suspected Mast Cell Activation Syndrome. J Allergy Clin Immunol Pract 2019;7:1125-33.e1.
86. Valent P, Bonadonna P, Hartmann K, Broesby-Olsen S, Brockow K, Butterfield JH, et al. Why the 20% + 2 Tryptase Formula Is a Diagnostic Gold Standard for Severe Systemic Mast Cell Activation and Mast Cell Activation Syndrome. Int Arch Allergy Immunol 2019;180:44–51.
87. Weiler CR, Austen KF, Akin C, Barkoff MS, Bernstein JA, Bonadonna P, et al. AAAAI Mast Cell Disorders Committee Work Group Report: Mast cell activation syndrome (MCAS) diagnosis and management. J Allergy Clin Immunol 2019;144:883–96.
88. Hogan AD, Schwartz LB. Markers of mast cell degranulation. Methods 1997;13:43-52.
89. Ebo DG, de Puysseleyr L, Van Gasse AL, Elst J, van der Poorten ML, Faber MA, Mertens C, Van Houdt M, Hagendorens MM, Sermeus L, Vitte J, Michel M, Garvey LH, Castells MC, Tacquard C; Mertes PM, Sabato V. Mast cell activation during suspected perioperative hypersensitivity: a need for paired samples. Submitted, in revision.
90. Malinovsky JM, Decagny S, Wessel F, Guilloux L, Mertes PM. Systematic follow-up increases incidence of anaphylaxis during adverse reactions in anesthetized patients. Acta Anaesthesiol Scand 2008;52:175-81.
91. Garvey LH, Melchiors BB, Ebo DG, Mertes P-M, Krøigaard M. Medical algorithms: Diagnosis and investigation of perioperative immediate hypersensitivity reactions. Allergy 2020;75:2139-42.
92. Hopkins PM, Cooke PJ, Clarke RC, Guttormsen AB, Platt PR, Dewachter P, et al. Consensus clinical scoring for suspected perioperative immediate hypersensitivity reactions. Br J Anaesth 2019;123:e29-e37.

93. Simons FER, Ebisawa M, Sanchez-Borges M, Thong BY, Worm M, Tanno LK, et al. 2015 update of the evidence base: World Allergy Organization anaphylaxis guidelines. World Allergy Organ J 2015;8:1–16.
94. Lieberman P, Nicklas RA, Oppenheimer J, Kemp SF, Lang DM, Bernstein DI, et al. The diagnosis and management of anaphylaxis practice parameter: 2010 update. J Allergy Clin Immunol. 2010 Sep;126(3):477-80.e1-42. doi: 10.1016/j.jaci.2010.06.022.
95. Simons FE, Ardusso LR, Bilò MB, El-Gamal YM, Ledford DK, Ring J, et al. World allergy organization guidelines for the assessment and management of anaphylaxis. World Allergy Organ J. 2011 Feb;4(2):13-37. doi: 10.1097/WOX.0b013e318211496c.
96. Muraro A, Roberts G, Worm M, Bilò MB, Brockow K, Fernández Rivas M, et al. Anaphylaxis: guidelines from the European Academy of Allergy and Clinical Immunology. Allergy. 2014 Aug;69(8):1026-45. doi: 10.1111/all.12437
97. Egner W, Cook TM, Garcez T, Marinho S, Kemp H, Lucas DN, et al. Specialist perioperative allergy clinic services in the UK 2018: Results from the Royal College of Anaesthetists Sixth National Audit Project (NAP6) investigation of perioperative anaphylaxis. Clin Exp Allergy 2018;48(7):846–61.
98. Ebo DG, Van Gasse AL, Decuyper II, Uyttebroek A, Sermeus LA, Elst J, et al. Acute Management, Diagnosis, and Follow-Up of Suspected Perioperative Hypersensitivity Reactions in Flanders 2001-2018. J Allergy Clin Immunol Pract 2019;7:2194-204.e7.
99. Kristensen T, Vestergaard H, Bindslev-Jensen C, Mortz CG, Kjaer HF, Ollert M, et al. Prospective evaluation of the diagnostic value of sensitive KIT D816V mutation analysis of blood in adults with suspected systemic mastocytosis. Allergy 2017;72:1737–43.
100. Giannetti MP, Akin C, Hufdhi R, Hamilton MJ, Weller E, van Anrooij B, et al. Patients with mast cell activation symptoms and elevated baseline serum tryptase have unique bone marrow morphology. J Allergy Clin Immunol 2020 Nov 25; S0091-6749(20)31633-X. doi: 10.1016/j.jaci.2020.11.017.
101. Egner W, Sargur R, Shrimpton A, York M, Green K. A 17-year experience in perioperative anaphylaxis 1998-2015: harmonizing optimal detection of mast cell mediator release. Clin Exp Allergy 2016;46:1465–73.
102. De Schryver S, Halbrich M, Clarke A, La Vieille S, Eisman H, Alizadehfar R, et al. Tryptase levels in children presenting with anaphylaxis: Temporal trends and associated factors. J Allergy Clin Immunol 2016;137:1138–42.

712 103. Castells M. Diagnosis and management of anaphylaxis in precision medicine. J Allergy Clin
713 Immunol 2017;140:321–33.
714 104. Valent P, Sperr WR, Sotlar K, Reiter A, Akin C, Gotlib J, et al. The serum tryptase test: an emerging
715 robust biomarker in clinical hematology. Expert Rev Hematol 2014;7:683–90.
716 105. Blossom DB, Kallen AJ, Patel PR, Elward A, Robinson L, Gao G, et al. Outbreak of adverse reactions
717 associated with contaminated heparin. N Engl. J Med 2008;359:2674-84.
718 106. Hakim RM, Breillatt J, Lazarus JM, Port FK. Complement activation and hypersensitivity reactions
719 to dialysis membranes. N Engl J Med 1984;311: 878-82.
720 107. Wolfler A, De Silvestri A, Camporesi A, Ivani G, Vittori A, Zadra N, et al. Pediatric anesthesia
721 practice in Italy: a multicenter national prospective observational study derived from the APRICOT
722 Trial. Minerva Anestesiol 2020;86:295–303.
723 108. Wakimoto M, Miller R, Kim SS, Uffman JC, Nafiu OO, Tobias JD, et al. Perioperative anaphylaxis
724 in children: a report from the Wake-Up Safe collaborative. Paediatr Anaesth 2020; doi:
725 10.1111/pan.14063
726 109. Khaleva E, Franz A, Garvey LH, Jay N, Ylescupidez A, Bahnson HT, et al. Perioperative anaphylaxis
727 in children: Etiology, time sequence, and patterns of clinical reactivity. Pediatr Allergy Immunol
728 2020;31:85–94.
729 110. Karila C, Brunet-Langot D, Labbez F, Jacqmarcq O, Ponvert C, Paupe J, et al. Anaphylaxis during
730 anesthesia: results of a 12-year survey at a French pediatric center. Allergy 2005;60:828–34.
731 111. Fieler M, Eich C, Becke K, Badelt G, Leimkühler K, Messroghli L, et al. Metamizole for
732 postoperative pain therapy in 1177 children: A prospective, multicentre, observational,
733 postauthorisation safety study. Eur J Anaesthesiol 2015;32:839–43.
734 112. Stepanovic B, Sommerfield D, Lucas M, von Ungern-Sternberg BS. An update on allergy and
735 anaphylaxis in pediatric anesthesia. Paediatr Anaesth 2019;29:892–900.
736 113. Hepner DL, Castells M, Mouton-Faivre C, Dewachter P. Anaphylaxis in the clinical setting of
737 obstetric anesthesia: a literature review. Anesth Analg 2013;117:1357–67.
738 114. McCall SJ, Bonnet MP, Äyräs O, Vandenberghe G, Gissler M, Zhang WH, et al. Anaphylaxis in
739 pregnancy: a population-based multinational European study. Anaesthesia. 2020
740 Nov;75(11):1469-1475. doi: 10.1111/anae.15069.
741 115. Simons FER, Schatz M. Anaphylaxis during pregnancy. J Allergy Clin Immunol 2012;130:597–606.

116. Boehm T, Reiter B, Ristl R, Petroczi K, Sperr W, Stimpfl T, et al. Massive release of the histamine-degrading enzyme diamine oxidase during severe anaphylaxis in mastocytosis patients. Allergy 2019;74:583–93.
117. Clark SL, Romero R, Dildy GA, Callaghan WM, Smiley RM, Bracey AW, *et al*. Proposed diagnostic criteria for the case definition of amniotic fluid embolism in research studies. *Am J Obstet Gynecol* 2016;215:408-12.
118. Stafford IA, Moaddab A, Dildy GA, Klassen M, Berra A, Watters C, *et al*. Amniotic fluid embolism syndrome: analysis of the Unites States International Registry. *Am J Obstet Gynecol MFM* 2020;2:100083.
119. Yunginger JW, Nelson DR, Squillace DL, Jones RT, Holley KE, Hyma BA, et al. Laboratory investigation of deaths due to anaphylaxis. J Forensic Sci 1991;36:857–65.
120. Garland J, Ondruschka B, Da Broi U, Palmiere C, Glenn C, Morrow P, et al. Differences Between Central and Peripheral Postmortem Tryptase Levels. Am J Forensic Med Pathol 2020 Oct 7; doi: 10.1097/PAF.0000000000000623. Online ahead of print
121. Edston E, Eriksson O, van Hage M. Mast cell tryptase in postmortem serum-reference values and confounders. Int J Legal Med 2007;121:275–80.
122. Garland J, Ondruschka B, Da Broi U, Palmiere C, Tse R. Post mortem tryptase: A review of literature on its use, sampling and interpretation in the investigation of fatal anaphylaxis. Forensic Sci Int 2020;314:110415.
123. Laroche D, Gomis P, Gallimidi E, Malinovsky J-M, Mertes PM. Diagnostic value of histamine and tryptase concentrations in severe anaphylaxis with shock or cardiac arrest during anesthesia. Anesthesiology 2014;121:272–9.
124. Edston E, van Hage-Hamsten M. Mast cell tryptase and hemolysis after trauma. Forensic Sci Int 2003;131:8–13.

Figure legends

Figure 1. Box 1. Definitions, nomenclature, and mechanisms of perioperative hypersensitivity and anaphylaxis. References: 1;4;6.

An overview of the definitions, nomenclature, and mechanisms of immediate perioperative hypersensitivity reactions is provided. Perioperative anaphylaxis is defined as a severe, life-threatening immediate perioperative hypersensitivity involving at least two organs, or circulatory or respiratory compromise.

Figure 2. Graphical summary of events and considerations in suspected perioperative hypersensitivity and anaphylaxis. References: 6-7;18-19.

ACE, angiotensin converting enzyme; a-Gal, alpha-galactose; IgE, immunoglobulin E; NMBA, neuromuscular blockers; POH, perioperative hypersensitivity reaction.

Figure 3. Clinical vignette

3a, perioperative presentation and management; 3b, examples of diagnostic assessment and recommendations for future anesthesia. References: 6;12;18;20-21.

BAT, basophil activation test; IgE, immunoglobulin E; NMBA, neuromuscular blockers; POH, perioperative hypersensitivity; sAT, serum acute tryptase; sBT, serum baseline tryptase; ST, skin tests

Figure 4. Historical overview of tryptase as a biomarker.

Tryptase was discovered in 1981. It is a biomarker of mast cell activation and burden, with applications in anaphylaxis and other immediate hypersensitivity reactions, mast cell disorders, hereditary α-tryptasemia, among others. Paired acute and baseline total tryptase determination is recommended for the diagnosis of mast cell activation in MCAS and perioperative settings.

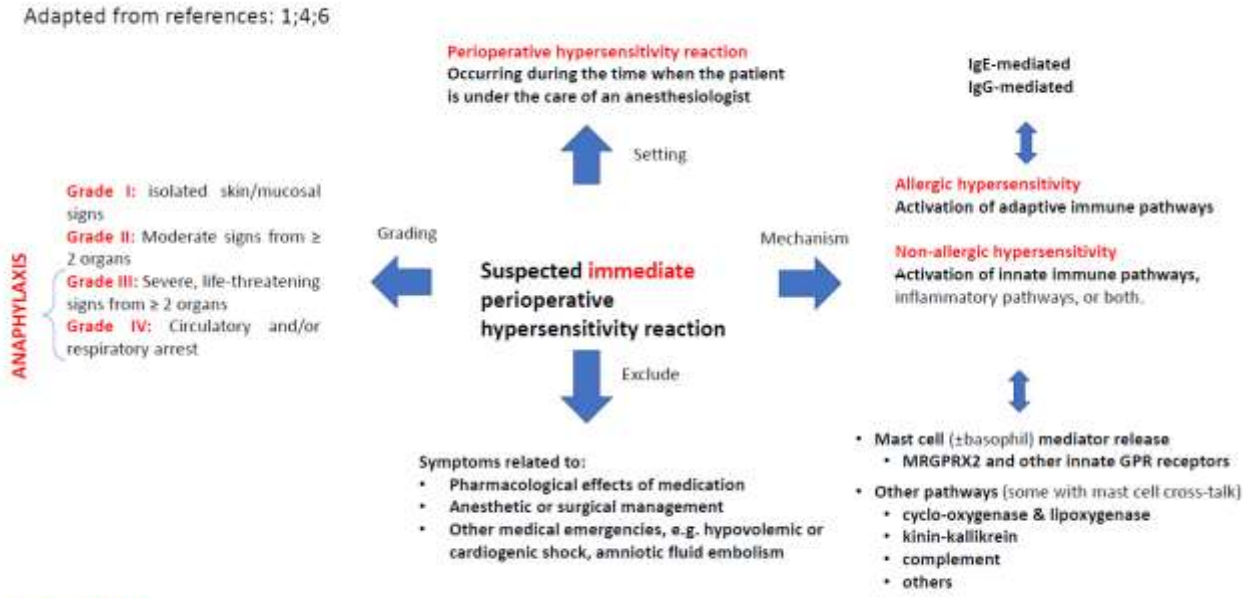
References: 6;58;67-68;70;73;75-87.

a, anti; Ab, antibody; ALP, alkaline phosphatase; CNV, copy number variation; FDA, Food and Drug Administration; Gal, beta-galactosidase; HaT, hereditary α –tryptasemia; hTry, human tryptase; mAb, monoclonal Ab; MCAS, mast cell activation syndrome; mu, murine; n, purified; r, recombinant; sBT, serum baseline tryptase level; sAT, serum acute tryptase level; WHO, World Health Organization.

Figure 5. Recommended algorithm for tryptase sampling during perioperative hypersensitivity and anaphylaxis.

This figure focuses on practical guidance for tryptase sampling during perioperative hypersensitivity including technical advice, pitfall avoidance and the mandatory referral to allergist.

Figure 1 = Box 1. Definitions, nomenclature, and mechanisms of perioperative hypersensitivity and anaphylaxis

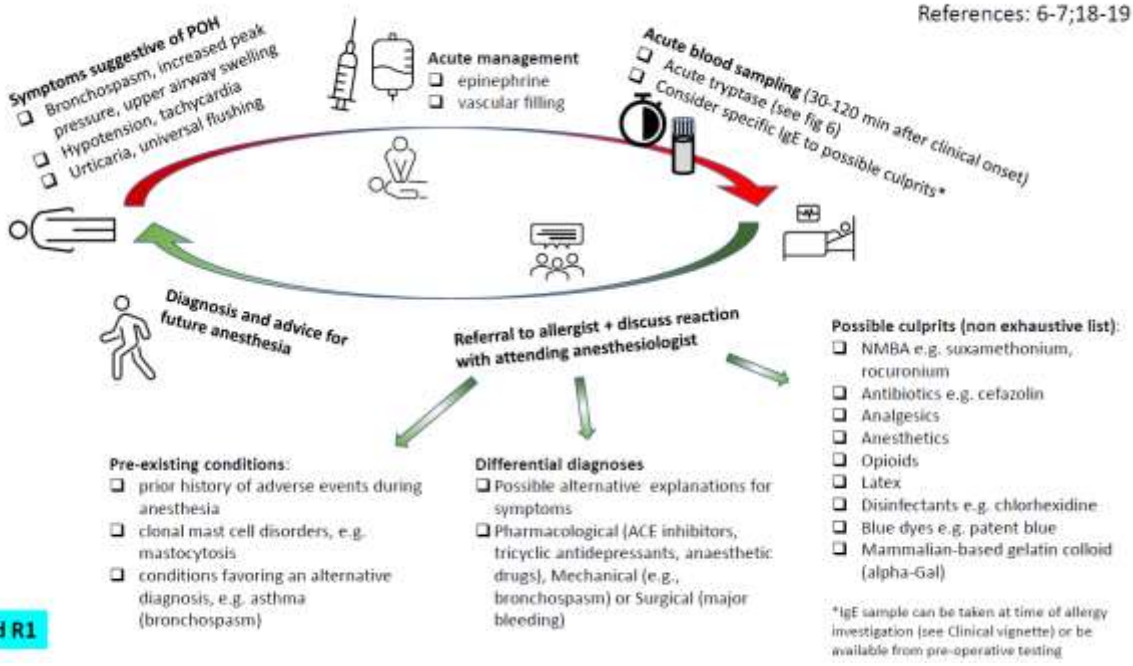


Revised R1

807

Figure 2. Graphical resume of events and considerations in suspected POH

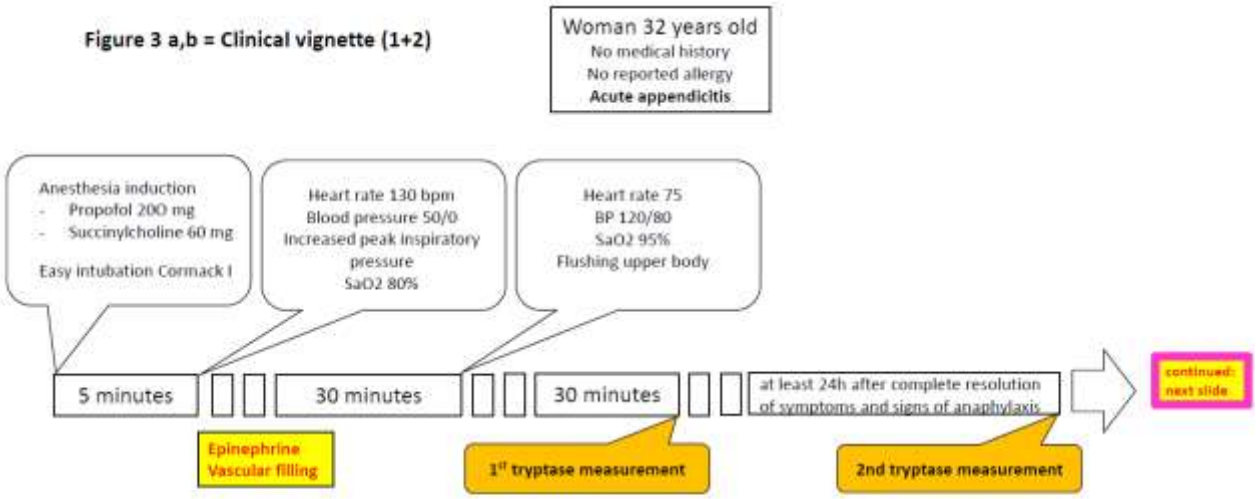
References: 6-7;18-19



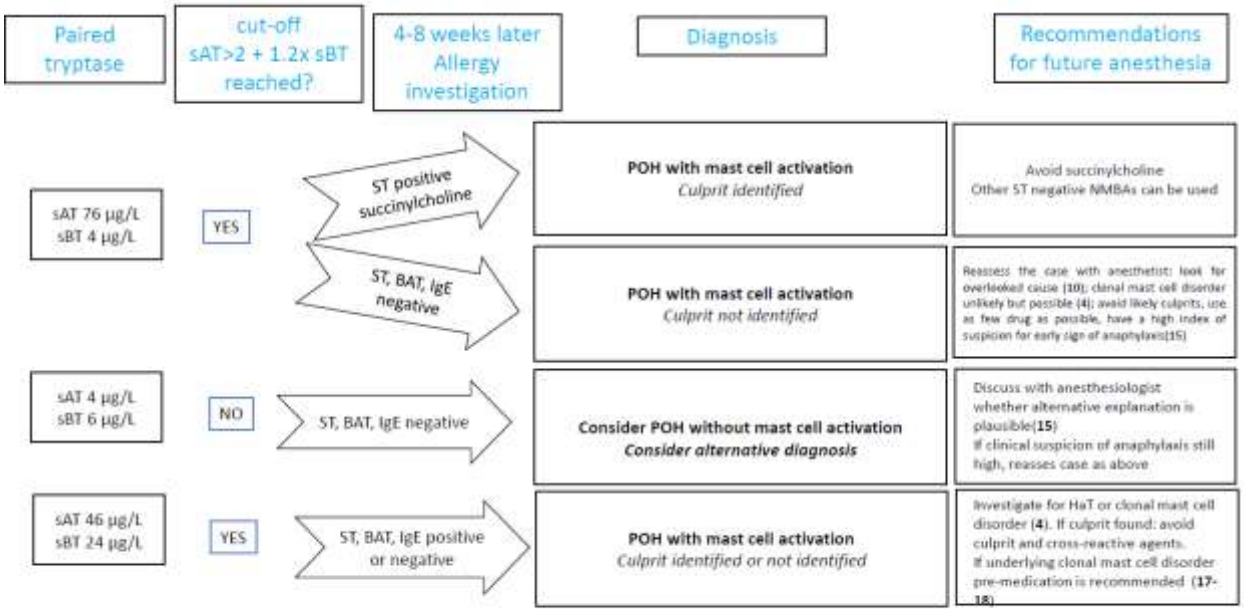
Revised R1

808

Figure 3 a,b = Clinical vignette (1+2)



809

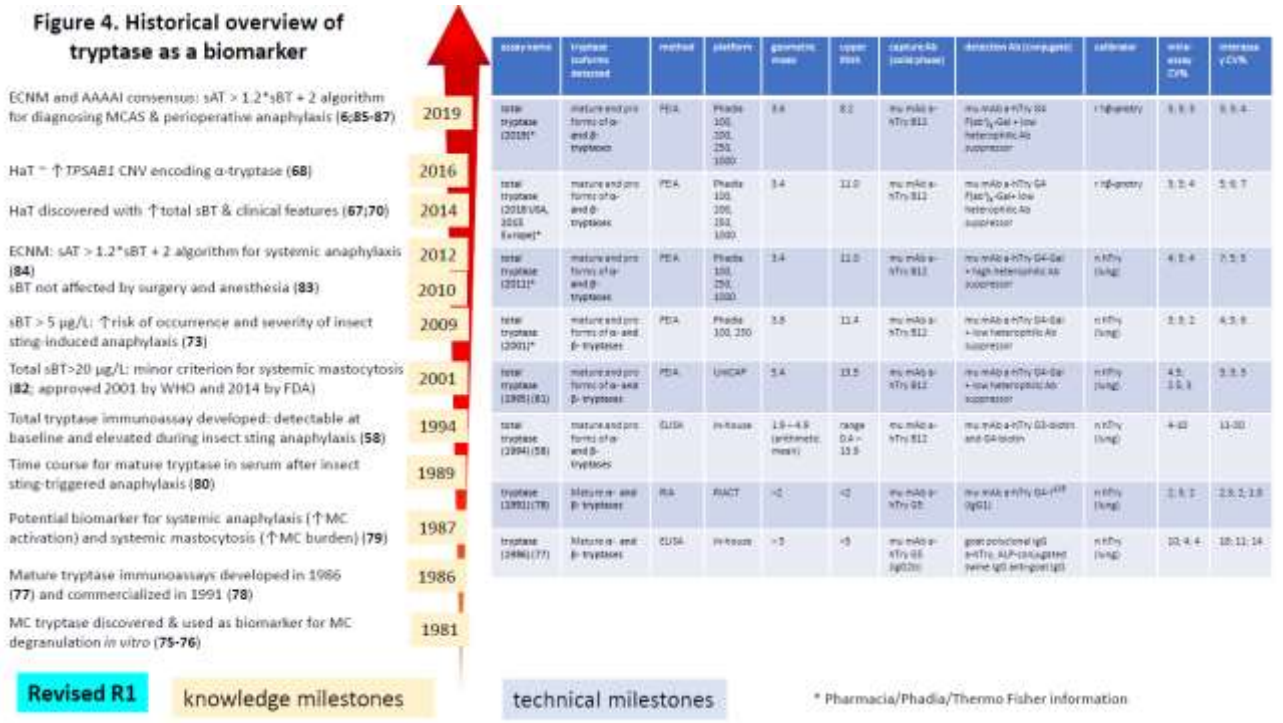


*A normal sBT does not exclude an underlying clonal mast cell disorder

References: 6; 12;18;20-21 **Revised R1**

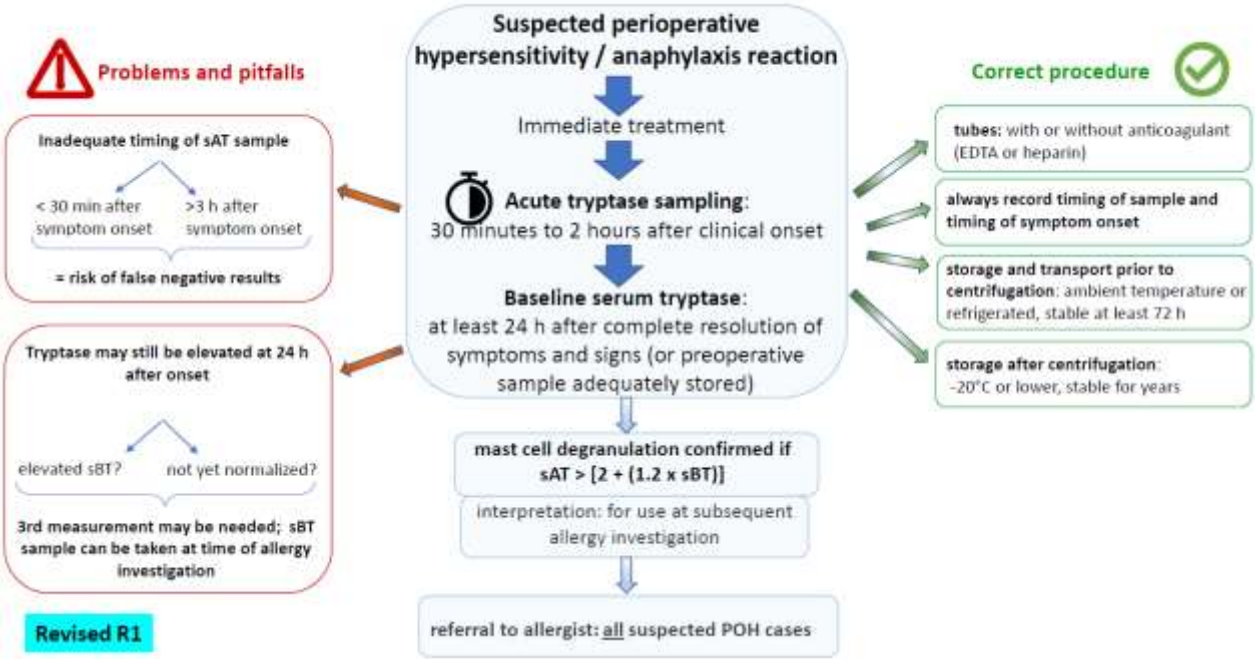
810

Figure 4. Historical overview of tryptase as a biomarker



811

Figure 5. Recommended strategy for tryptase sampling during POH



812