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**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.

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**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**

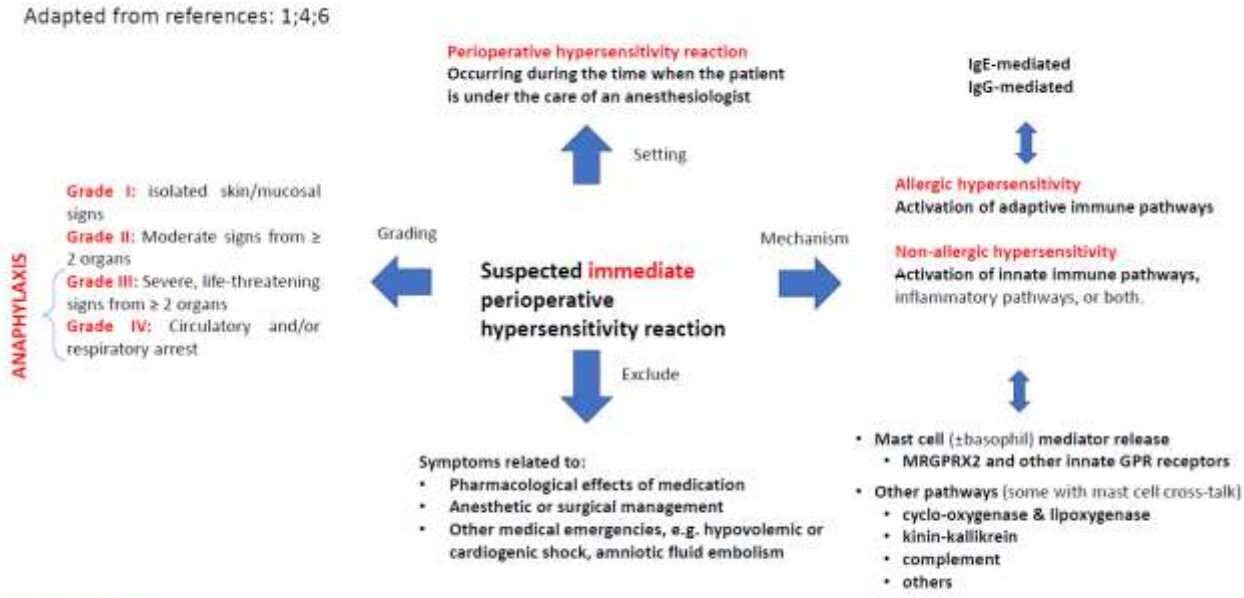


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**Figure 2. Graphical resume of events and considerations in suspected POH**

References: 6-7;18-19

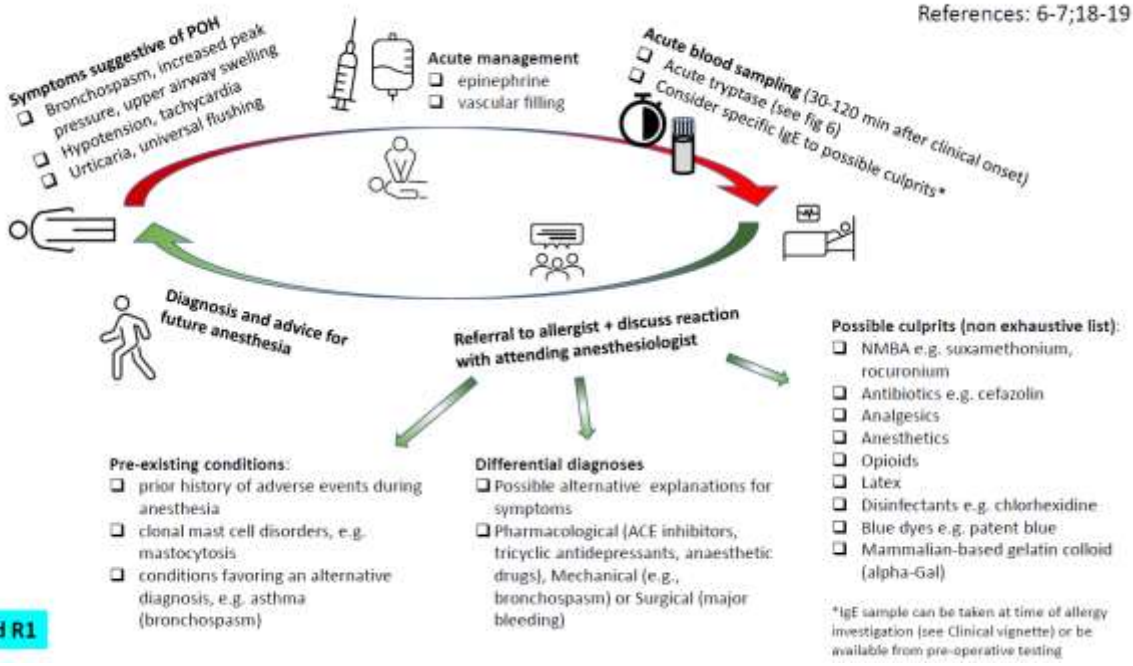


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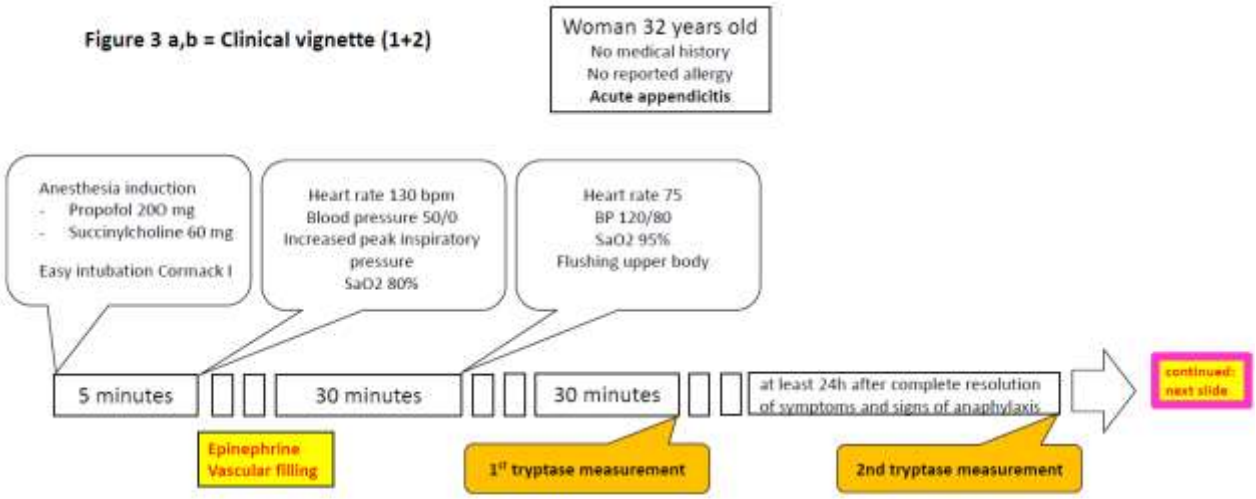
**Figure 3 a,b = Clinical vignette (1+2)**

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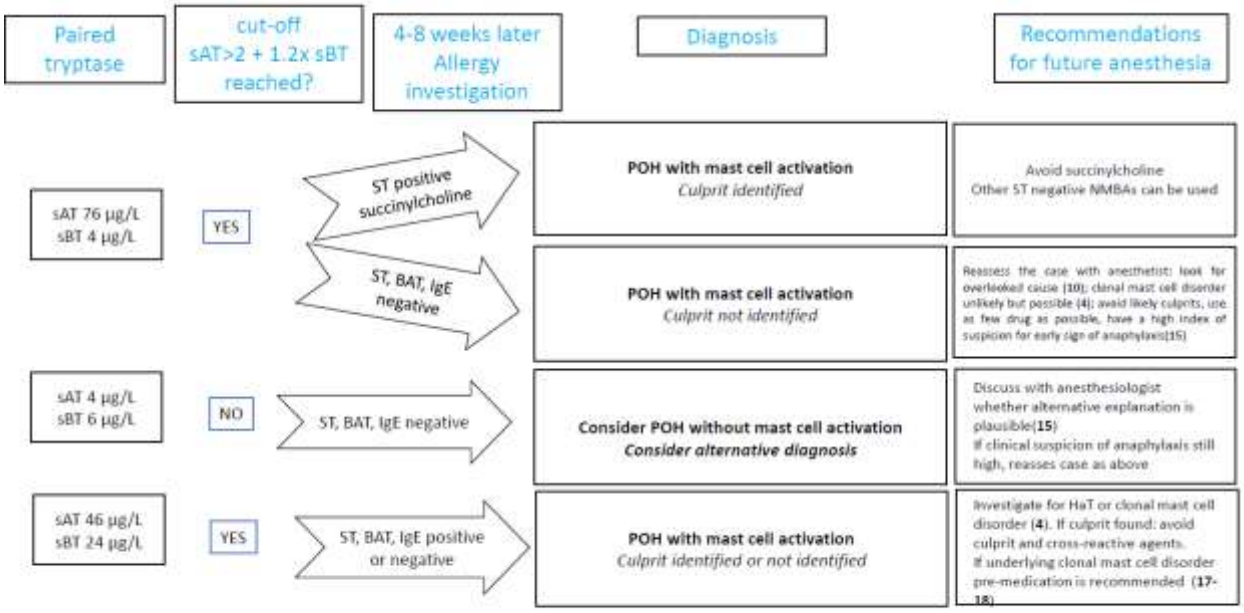


*A normal sBT does not exclude an underlying clonal mast cell disorder

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

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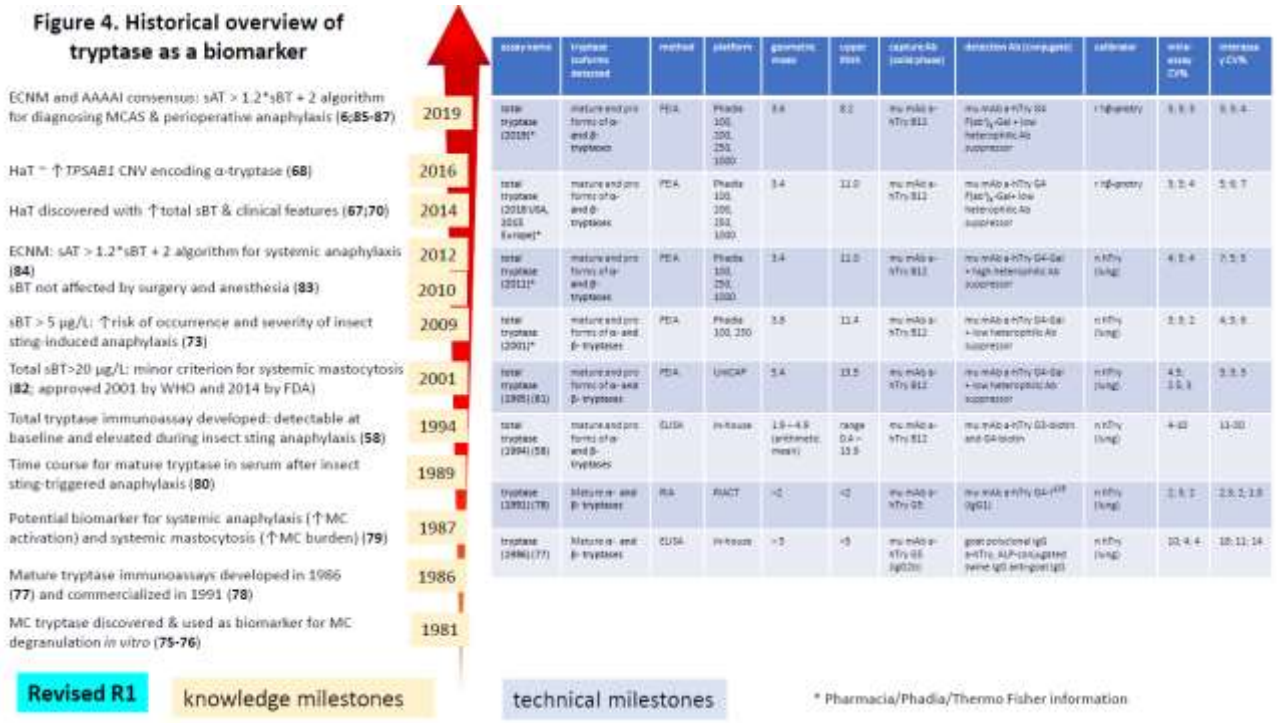
## Figure 4. Historical overview of tryptase as a biomarker

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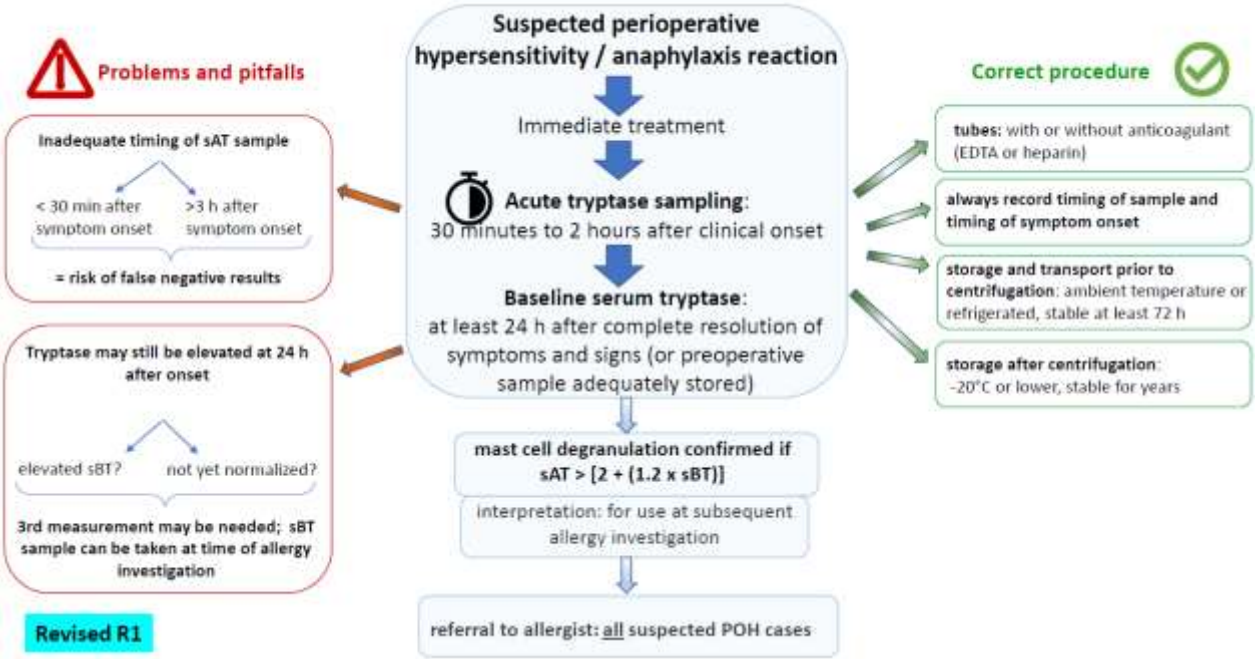
## Figure 5. Recommended strategy for tryptase sampling during POH

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