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Why the 20% + 2 Tryptase Formula Is a Diagnostic Gold Standard for Severe Systemic Mast Cell Activation and Mast Cell Activation Syndrome

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Abstract
Mast cell activation syndrome (MCAS) is a condition characterized by recurrent episodes of clinically relevant, systemic, severe reactions to mast cell (MC)-derived mediators released in the context of anaphylaxis or another acute MC-related event. It is important to document MC involvement in these reactions in order to establish the diagnosis MCAS. The most specific and reliable marker of systemic MC activation is an acute and substantial event-related (transient) increase in the serum tryptase level over the individual's baseline value. However, the baseline level of tryptase varies depending on the underlying disease and the genetic background. For example, an estimated 3–5% of healthy individuals exhibit duplications or multiple copies of the *TPSAB1* gene encoding for alpha-tryptase, and over 30% of all patients with myeloid neoplasms, including mastocytosis, have elevated basal tryptase levels. Therefore, it is of utmost importance to adjust the event-related diagnostic (MCAS-confirming) increase in tryptase over the individual baseline in a robust approach. To address this challenge, the 20% + 2 formula was proposed by the consensus group in 2012. Since then, this approach has been validated in clinical practice by independent groups and found to be sound. In the current article, we discuss the emerging importance and value of the 20% + 2 formula in clinical practice and its role as a criterion of severe systemic MC activation and MCAS.

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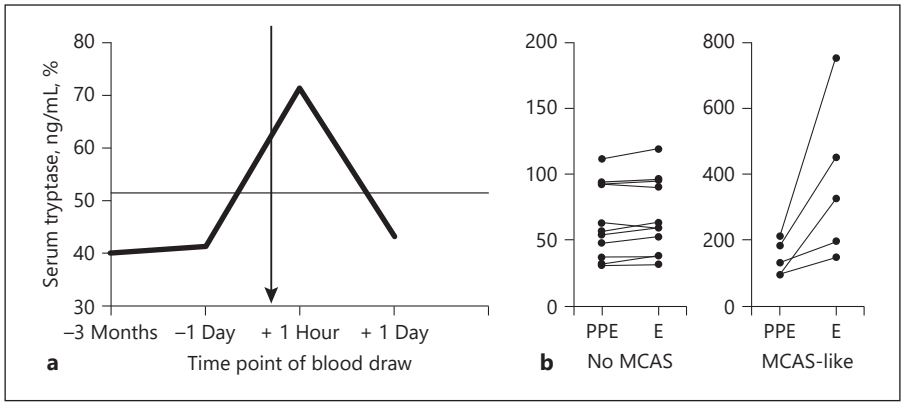

Fig. 1. a Total serum tryptase concentrations in a patient with indolent systemic mastocytosis and bee venom allergy. The patient developed anaphylaxis (dyspnea, hypotension, tachycardia) 15 min after subcutaneous injection of 0.3 µg bee venom immunotherapy. Whereas his basal tryptase levels were stable before the reaction (40.1, 41.2 ng/mL) and the value returned to 43.2 ng/mL 16 h after the reaction, the tryptase level in the serum obtained within 2 h after anaphylaxis increased to 71.3 ng/mL. This level exceeded the diagnostic threshold by plus 20% + 2 ng/mL (41.2 + 8.24 + 2 ng/mL = 51.44 ng/mL) and thus qualified as MCAS criterion. **b** Patients with mastocytosis with mild clinical symptoms not resembling MCAS (no MCAS – left panel) and those with severe clinical symptoms resembling MCAS (MCAS-like – right panel) were examined for their serum tryptase levels post or prior to the event and at or shortly after the event (E). As visible, a diagnostic increase in tryptase was only captured in those with a severe anaphylactic (MCAS-like) event (E). MCAS, mast cell activation syndrome; PPE, post or prior to the event.

Introduction

Mast cells (MCs) are tissue-resident multifunctional effector cells of the immune system [1–3]. These cells produce not only an array of pro-inflammatory mediators and cytokines, including histamine, cysteinyl leukotrienes and prostaglandins, but also various proteases and certain proteoglycans such as heparin [1–3]. During and following anaphylactic degranulation, usually triggered through IgE receptor cross-linking by an allergen, MC release their granular mediators. MC activation and degranulation can also be induced by other triggers or by an IgE-independent hypersensitivity reaction [1–6].

Tryptases are serine proteases that are preferentially produced and stored in MC [7, 8]. In fact, tryptases account for >20% of the total protein content in tissue MC [9]. Whereas mature tryptases are mostly stored in the heparin-containing metachromatic granules of human MC, the precursor forms (pro-tryptases) are released spontaneously and constantly from resting MC [10, 11]. Based on a constant release rate in the steady state and the chemical and biological stability of tryptases, the resulting basal serum tryptase level is remarkably consistent in healthy individuals. The serum tryptase level also remains stable in various reactive processes. However, during and shortly after a severe anaphylactic event where MC release large amounts of tryptase, the serum tryptase level increases substantially over the individual's baseline (Fig. 1) [12–17]. Thereafter, the tryptase level returns to baseline, which may take several hours (up to 24 h), providing a reliable diagnostic window of about 2–4 h for laboratory investigations [18]. Although other MC-derived chemical mediators and their metabolites, like histamine, prostaglandin D2 (PGD2), and under certain circumstances heparin, may also serve as indicator(s) of MC activation, tryptase has been repeatedly described as the most specific and reliable biomarker of severe systemic MC activation in daily practice [12–18]. A summary of MC-related biomarkers that have been considered (and may be used) for the detection and quantification of MC activation in patients with anaphylaxis is provided in Table 1.

Systemic MC activation is commonly found in severe anaphylactic reactions [1–6]. In most patients, an IgE-dependent allergy or another hypersensitivity disorder is identified. In severe cases, a MC activation syndrome (MCAS) may be diagnosed [19–22]. Patients with MCAS fulfill specific criteria (see below) and are classified based on the underlying disease. Thus, MCAS is divided into (i) primary (mono/clonal) MCAS, where monoclonal *KIT*-mutated MC can be detected, (ii) secondary MCAS, where an allergic or other reactive inflammatory disease process may be identified as a trigger of MC activation, and (iii) idiopathic MCAS, where neither MC clonality

Table 1. Biomarkers indicating systemic severe MC activation in patients

Biomarker	Specificity for MCs	Sensitivity in anaphylaxis	Commonly used in daily practice
Tryptase	++*	+**	++
Plasma histamine	+/–		+
Urinary histamine metabolites***	+/–	++	++
PGD2 metabolites****	+/–	++	+
Urinary cysLT levels	–/+	++	+/–
Heparin	+++	–/+*****	–
DAO	–******	++	–

* Basophils express very low amounts of tryptase, but MCs are a primary and major source of the enzyme.

** The relatively low sensitivity of tryptase qualifies as a biomarker of massive MC activation and thus as a criterion of MCAS.

*** Relevant 24-h urinary histamine metabolites include N-methylhistamine and N-methylimidazoleacetic acid.

**** Among PGD2 metabolites, the most commonly measured substance is urinary 11β-prostaglandinF2α.

***** An increase in heparin is usually not measurable during an anaphylactic episode, unless the burden of MCs is very high (like in MC leukemia).

****** So far it is not known whether the increased DAO levels measured in patients during anaphylaxis are derived from MCs or (also) other cell types.

cysLT, cysteinyl leukotriene; DAO, diamino-oxidase; MCAS, mast cell activation syndrome; PGD2, prostaglandin D2; MC, mast cell.

nor an underlying allergic or other inflammatory or toxic disease process is diagnosed [19–22].

MCAS criteria have been widely accepted and applied in daily practice, mostly in the context of sudden hypotension and shock or other signs of severe anaphylaxis, but also in the context of less severe symptoms where these criteria may not be fulfilled (exclusion of MCAS) [23, 24]. Based on the recommendations of the EU/US consensus group, MCAS is diagnosed when the following criteria apply: (i) documented recurrent episodic occurrence of typical systemic symptoms that are produced by MC mediators and involve at least 2 organ systems, (ii) an event-related transient elevation of the serum tryptase level by at least 20% over the individual baseline plus 2 ng/mL absolute (e.g., from 15 ng/mL to at least + 3 + 2 ≥20 ng/mL or from 41.2 ng/mL to at least + 8.24 + 2 ≥52 ng/mL; Fig. 1) within a 2–4 h window after the reaction, and (iii) a documented clinically meaningful response to drugs that either target MC-derived mediators (e.g., H1 antihistamines or antileukotrienes) and/or suppress MC activation (e.g., cromoglycate, ketotifen, or omalizumab) [19, 21, 22].

Although these criteria are generally accepted and their application has been validated, there remains an ongoing discussion on their use and usefulness in daily practice. One concern relates to the 20% + 2 formula mentioned above. In particular, many users are not familiar with this approach and ask questions related to this formula and why it has been proposed in the MCAS context.

In the current article, we discuss these issues and explain why, where, when, and how this MCAS criterion is applied in routine medical practice and why it remains a most important diagnostic standard.

Scientific and Conceptual Basis of the 20% + 2 Formula

During the 2010 Working Conference on MC disorders where MCAS criteria were discussed and formulated, one of the deliberation points was the minimal increase in serum tryptase over the individual's baseline that would meet the criterion of a significant systemic MC activation and would thus count as a diagnostic marker of MCAS [21]. In fact, although it was clear that tryptase is the biomarker of choice to define anaphylaxis and thus MCAS based on previous literature data and experience in specialized centers [12–18], a diagnostic threshold was missing.

The challenge was to define a minimal increase in tryptase over the individual's baseline level that could be employed for all conditions and cases, namely, individu-

als with a low basal serum tryptase level (1–5 ng/mL), those with a slightly elevated basal tryptase (12–50 ng/mL), cases with markedly elevated basal tryptase (>50–200 ng/mL), and patients with very high tryptase levels (> 200 ng/mL). Employing only an absolute amount of extra tryptase as criterion (e.g., plus extra 20 ng/mL) would fail in those who have very low basal tryptase levels (1–5 ng/mL) and in those with systemic mastocytosis (SM) with a tryptase level exceeding 100 or 200 ng/mL. Defining only a relative increase in tryptase as a criterion (e.g., by 10 or 20%) would fail in those who have low enzyme levels (below 10 ng/mL; e.g., an increase from 5 to 5.7 or even 6 cannot be regarded as a marker of severe systemic anaphylaxis).

It is also important to note that a persistently elevated serum tryptase concentration, like found in SM, is not indicative of MC activation but reflects the elevated body burden of MC. In other cases, a constantly increased basal release or an extra amount of alpha tryptase gene copies can lead to a persistently elevated basal tryptase – again, however, such persistently elevated tryptase is not an indication of systemic MC activation or MCAS.

After in-depth deliberations and based on shared practical experience, the faculty did come up with a consensus proposal and put forth the 20% + 2 formula during the Working Conference [21]. This formula defines a minimal diagnostic increase in tryptase over the individuals' baseline that qualifies as solid indication and thus as criterion of severe systemic MC activation in all cohorts of patients, including those with very low or normal basal serum tryptase, those with slightly elevated basal tryptase, and those with highly elevated basal serum tryptase. The 20% + 2 formula was subsequently validated against the available literature and all retrospective cases published, and the formula was found to be applicable and robust both in the context of allergy and in the context of mastocytosis (K.B., L.B., and P.V., unpublished observation) – see also Figure 1. Later, the 20% + 2 formula was further validated in independent case series – see below. Figure 1 shows typical examples of patients with anaphylaxis where the 20% + 2 formula worked out well.

Conditions Associated with an Elevated Baseline Serum Tryptase Level

The first question concerning the 20% + 2 formula is: how frequently is an elevated basal serum tryptase level (>11.4 ng/mL, as defined in the commercial assay) detected in daily practice in healthy individuals, in patients diagnosed with allergic/atopic disorders, chronic inflammatory diseases, hematologic disorders, and other diseases of internal medicine? The answer is: when taking the 11.4 threshold as denominator, an increased basal tryptase level is found frequently in otherwise healthy individuals (roughly 5–10% of the general population), in diverse reactive states, and in hematologic disorders, especially in myeloid malignancies (roughly 20–30%; Table 2) [25–31]. Thus, a number of genetic, inflammatory, and neoplastic conditions are associated with an increased basal serum tryptase level. These include tryptase gene replications such as hereditary alpha tryptasemia; chronic infections, such as helminth infections; renal insufficiency – especially hemodialysis patients; and myeloid neoplasms, including myelodysplastic syndromes, SM, acute myeloid leukemia, chronic myeloid leukemia (CML), and chronic eosinophilic leukemia [25–33].

Depending on the underlying condition, basal tryptase levels can be slightly elevated, substantially elevated, or even highly elevated (Table 2). For example, most cases of hereditary alpha tryptasemia have slightly elevated tryptase (12–30 ng/mL) [30–33]. However, the tryptase level increases with gene copy number and can be up to 100 ng/mL in those who have multiple tryptase gene copies (P.V., P.B., and J.J.L., unpublished observation and [33]).

In patients with myeloid neoplasms, the range of tryptase is also broad (Table 2). In most patients with myelodysplastic syndromes or myeloproliferative syndromes, such as CML or chronic eosinophilic leukemia, serum tryptase levels are slightly elevated [27–29]. However, in some patients with acute myeloid leukemia, especially those with inv (16) or *t*(8;21), basal serum tryptase levels may increase to 100, over 200, or even 1,000 ng/mL (Table 2) [25]. Similarly, in patients with mastocytosis, especially in those who suffer from advanced SM, basal serum tryptase levels may exceed 200 or even 1,000 ng/mL [14, 16, 26]. Finally, as mentioned, there are a number of reactive conditions where serum tryptase levels are elevated, such as chronic kidney failure or chronic helminth infections [28, 34, 35]. All in all, the serum tryptase level can range substantially from subject to subject, both in the general, apparently healthy, population (due to genetic variability and gene copy number variation), in reactive inflammatory conditions, and in myeloid neoplasms. This variation in tryptase levels thus requires a flexible formula defining the minimal increase in tryptase during an MCAS screen in all instances.

Table 2. Conditions associated with an elevated basal serum tryptase level

Condition	Typical range of serum tryptase in ng/mL*	Increased risk for MCAS
Hereditary alpha tryptasemia	15–50**	+/–***
Cutaneous mastocytosis	5–15	+
Indolent systemic mastocytosis	15–200	++
Advanced systemic mastocytosis****	100–1,000	+
Myelodysplastic syndromes	10–50	–
Myeloproliferative neoplasms	10–100	–
Chronic eosinophilic leukemia	10–50	–
Chronic myeloid leukemia, CP	10–20	–
Chronic myeloid leukemia, AP	20–50	–
Acute myeloid leukemia	10–1,000	–
Chronic helminth infection	10–20	–***
Chronic renal failure	10–30	–

* Range of basal tryptase where a majority of all cases are found.

** In hereditary alpha tryptasemia, the tryptase level increases 7–15 ng/mL with each additional gene copy; in those with multiple copies (rare cases), tryptase levels can increase up to 100 ng/mL.

*** In these conditions, the incidence of severe anaphylaxis (MCAS) is not known although some reports suggest an increased risk.

**** Advanced systemic mastocytosis includes patients with aggressive systemic mastocytosis and MC leukemia. In these patients, the basal tryptase level may increase to >500 or even >1,000 ng/mL.

Score: ++, substantial risk to develop MCAS events one or more times per year despite therapy with anti-mediator-type drugs; +, increased risk to develop MCAS events one or more times over several years; +/–, MCAS events have been reported in individual patients, but the precise incidence is not clear; –, no increased risk to develop MCAS events compared to the general population (+/– allergic individuals).

MCAS, mast cell activation syndrome; CP, chronic phase; AP, accelerated phase; MC, mast cell.

The second question is whether one or more of these groups of individuals (healthy individuals or patients) with elevated basal tryptase have indeed an increased risk for developing MCAS. Here the answer is not simple. For example, there is some evidence that individuals with extra tryptase gene copies (hereditary alpha tryptasemia) are at increased risk to develop anaphylactic reactions, especially when a concomitant allergy is also present [30, 33, 36]. In the group of mastocytosis patients, the answer is clearer: in general, patients with SM have a higher risk to develop anaphylaxis and MCAS compared to otherwise healthy individuals or allergic patients without SM [37–44]. Patients with SM and a concomitant allergy have the highest risk to develop MCAS [40–42]. On the other hand, the risk of MCAS does not appear to increase with higher serum tryptase levels [43–46]. In fact, SM patients with a low basal tryptase level (below 30 ng/mL) can develop severe life-threatening anaphylaxis and thus MCAS in apparently a similar manner and frequency when compared to patients with SM with higher serum tryptase levels [43–47].

In myeloid neoplasms other than SM, the elevated tryptase level is often associated with an increase in immature MCs or immature basophils. Still, the risk for development of severe anaphylaxis is usually not greater than the risk found within the general population, thereby contrasting with the elevated risk in SM. However, CML patients can suffer from severe allergies and can rarely develop severe anaphylactic reactions resembling MCAS (P.V., unpublished observation). Whether in these patients, the large number of basophils play a pathologic role remains unknown.

Validation of the 20% + 2 Formula in Clinical Practice

Over the past few years, the 20% + 2 formula has been validated in patients with MC disorders as well as in patients without mastocytosis. In patients with SM, the 20% + 2 formula is a robust approach that safely discriminates between an anaphylactic event and less severe forms of MC activation not fulfilling MCAS criteria, independent

of the variant of SM or the basal serum tryptase level (unpublished data and [48]). These data confirm previous studies that have shown that a substantial increase in tryptase is a reliable parameter to document MC activation during anaphylactic episodes [12, 16–18]. However, even in patients who do not suffer from an allergy or mastocytosis, an increase in the serum tryptase level is a most reliable biomarker of MC activation [13, 15, 49]. For example, it has been reported that the 20% + 2 formula is a robust equation for confirming suspected perioperative anaphylaxis during general anesthesia [49]. Moreover, the formula has been validated in children presenting to emergency departments with anaphylaxis [49]. By contrast, in cases with local MC activation or mild mediator-related symptoms, including a subset of food allergy-related reactions associated with GI tract exposure, the serum tryptase level remains near the individual's baseline [48–52].

The documentation of a severe systemic MC activation and thus MCAS has also therapeutic implications, especially when other differential diagnoses have to be considered. In fact, MCAS patients often need immediate intensive therapy, such as anti-histamines, glucocorticosteroids, and/or epinephrine. In addition, depending on the underlying disease, such patients often receive MC stabilizers, KIT-targeting drugs, or immunotherapy.

Are There Alternative Biochemical Parameters Available?

Apart from tryptase, a number of other MC-derived compounds may serve as suitable parameters to document severe reactions following systemic MC activation. These substances include, among others, histamine and its metabolites, PGD2 and its metabolites, and heparin [53–58]. However, with the exception of heparin, these mediators are less specific for MC compared to tryptase (Table 1). Methods to determine these mediators are also much less available through widely distributed commercial assays. Some of these mediators have to be measured in 24-h urine samples collected under specific guidelines, including dietary restrictions, in order to obtain reliable results [53–57]. In addition, it remains unclear and undefined what minimal diagnostic increase in histamine (metabolites) or PGD2 metabolites qualifies as a robust indication and thus criterion of severe systemic MC activation and MCAS. Moreover, age-specific normal levels have not been established. Finally, most of these markers increase in a number of different reactive conditions and also in cases with mild mediator-related symptoms. Therefore, these markers may qualify as criteria for less severe forms of MC activation or as indicators of targets of therapeutic intervention (PGD2), but not as a robust criterion for MCAS.

More recently, diamino-oxidase, a histamine-degrading enzyme, has been described as a marker that increases in MCAS patients and has a similar specificity and time course compared to serum tryptase levels [48]. However, a validated test is not generally available, and it remains unknown whether diamino-oxidase is expressed and released from MCs during an anaphylactic reaction and thus is specific for MCAS.

Summary and Future Perspectives

The baseline level of serum tryptase varies considerably among apparently healthy individuals based on their genetic background and other factors. For example, the basal tryptase level is higher in cases of hereditary alpha tryptasemia compared to healthy individuals without an increase in *TPSAB1* copy number. The basal tryptase level is also elevated and varies among patients with MC disorders and other myeloid neoplasms as well as in reactive (inflammatory) states. Since several of these conditions may also predispose for the development of MC activation-related events, it is of utmost importance to provide a valuable and simple tool to define a clinically meaningful, reliable increase of the tryptase level over the individual's baseline through which a substantial (diagnostic) MC activation is safely documented. The 20% + 2 consensus equation is a sound tool to measure a clinically relevant increase in tryptase independent of the baseline level, including individuals with normal/low tryptase (<10 ng/mL), those with slightly elevated tryptase (<50 ng/mL) and those with clearly or even massively elevated tryptase. On the other hand, the tryptase test and the 20% + 2 formula are also robust in that mild forms and local forms of MC activation are usually not captured through this formula. All in all, the 20% + 2 formula remains a recommended gold standard in the evaluation of severe anaphylaxis and MCAS and thus a valid criterion of MCAS available as a clinical diagnostic tool. In the light of the many referrals of patients, this criterion is of even greater contemporary importance, as it helps in the evaluation to determine whether a patient is suffering from true MCAS.

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Author Contributions

All authors contributed equally by discussing the available literature, unpublished data, and case reports. All authors contributed substantially writing and/or extensively reviewing parts of the manuscript. All authors approved the final version of the document.

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