

Incorporating Tryptase Genotyping Into the Workup and Diagnosis of Mast Cell Diseases and Reactions



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The measurement of mast cell tryptase levels in serum has found utility in the diagnosis and management of both clonal mast cell disorders and severe mast cell–dependent systemic reactions in the form of anaphylaxis. A more recent discovery is that a majority of individuals with elevated basal serum tryptase levels have increased germline *TPSAB1* gene copy number encoding α -tryptase. This genetic trait is referred to as hereditary α -tryptasemia (H α T) and affects nearly 6% of the general population. In clinical practice, the presence or absence of H α T should thus now be determined when defining what constitutes an abnormal serum tryptase level in the diagnosis of mastocytosis. Further, as rises in serum tryptase levels are used to support the diagnosis of systemic anaphylaxis, variability in baseline serum tryptase levels should be factored into how significant a rise in serum tryptase is required to confirm the diagnosis of a systemic allergic reaction. In practicality, this dictates that symptomatic individuals undergoing evaluation for a mast cell–associated disorder or reaction with a baseline serum tryptase level exceeding 6.5 ng/mL should be considered for tryptase genotyping in order to screen for H α T. This review provides detailed information on how to use the results of such testing in the diagnosis and management of both mastocytosis

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Systemic mastocytosis (SM) is an acquired clinical disorder in which the expansion of clonal mast cells frequently occurs in association with the recurrent somatic gain-of-function pathogenic variant p.D816V in stem cell factor receptor *KIT*, leading to unrestrained proliferation of mast cell progenitors and augmented clinical symptoms of mast cell reactivity.¹ This rare condition is often associated with elevated baseline or basal serum tryptase (BST) levels that positively correlate with mast cell burden,^{2,3} which has resulted in the clinical use of this biomarker as a minor criterion for the clinical diagnosis of SM when elevated above 20 ng/mL.⁴ However, population-based studies have suggested that the prevalence of individuals with BST ≥ 20 ng/mL may approach 1% to 2% of all individuals.^{5,6} In 2014, it was first discovered that elevated BST levels were dominantly inherited in families.⁷ Subsequent studies have since demonstrated that such elevations result from increased germline *TPSAB1* gene copy number encoding α -tryptase,⁸ a genetic trait referred to as hereditary α -tryptasemia (H α T), accounting for most individuals with elevated BST in the general population.⁹ This review will examine the genetics of human tryptases, their impacts on BST levels, and how H α T can modify clinical phenotypes in SM. Finally, we will discuss how this knowledge may be leveraged to aid in the workup of individuals with elevated BST levels and applied to the clinical diagnoses of anaphylaxis and SM.

TRYPTASE AS A BIOMARKER OF CLONAL MYELOID NEOPLASMS AND ANAPHYLAXIS

Tryptase is an abundant serine protease, which in healthy individuals is almost exclusively generated by tissue mast cells and, to a much lesser extent, blood basophils.¹⁰ In part because expression of this protein is largely restricted to allergic effector cells and the half-life of this protein after release from mast cells after activation and degranulation is longer than other allergic mediators, tryptase has become a useful biomarker for anaphylaxis, where acute rises above set thresholds can support this clinical diagnosis.²

In addition to acute changes in total serum tryptase being useful for the identification of mast cell degranulation, increased baseline or BST levels have also been useful in the identification of clonal myeloid neoplasms.¹¹ Whereas acute changes in total serum tryptase after mast cell degranulation result from

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Abbreviations used

ACKD- Advanced chronic kidney disease
BST- Basal serum tryptase
CI- Confidence interval
ddPCR- Droplet digital polymerase chain reaction
H α T- Hereditary α -tryptasemia
PI- Prediction interval
SM- Systemic mastocytosis
TPSAB1- Tryptase α/β -1
TPSB2- Tryptase β -2
TPSD1- Tryptase δ -1
TPSG1- Tryptase γ -1

substantial release of mature enzymatically active tryptase tetramers, BST levels are dictated by the constitutive release of pro-tryptase zymogens by mast cells or neoplastic myeloid cells.^{12,13} The cardinal clonal disorder associated with elevated BST is SM, where most commonly, gain-of-function variants in the stem cell factor receptor *KIT* are associated with deregulated growth and expansion as well as the apparent enhanced excitability of this lineage.^{1,11} However, neoplastic myeloid cells, including blasts or immature basophils, have been shown to aberrantly express tryptases in a substantial number of patients with acute or chronic myeloid leukemia or other myeloid neoplasms, resulting in elevated BST levels.¹⁴

Although it has been recognized for some time that BST levels are elevated (>11.4 ng/mL) in approximately 6% of the general population,^{5,6,9} the relatively recent observation that these elevations are largely genetically determined¹⁵ has major implications for the use of BST in the evaluation and diagnosis of clonal myeloid neoplasms (discussed in more detail in later sections), and indeed what constitutes a “normal” BST level requires re-examination. Impacts on the evaluation of patients with suspected myeloid neoplasms would be most anticipated among individuals with SM—where a BST level above 20 ng/mL is a minor criterion for diagnosis. The caveat to this minor criterion has been that a concomitant hematological neoplasm precludes the use of this criterion because elevated BST in this context could be due to the second hematological neoplasm.

IMPACT OF BST LABILITY ON CONFIRMATION OF ANAPHYLAXIS AMONG INDIVIDUALS WITH ELEVATED LEVELS

Historically, the threshold for confirmation of the diagnosis of anaphylaxis has been accepted as a change of $\geq 20\% + 2$ ng/mL (20+2) over BST in an individual during an acute symptomatic event,¹⁶ where the acute level is measured within approximately 2 half-lives, or 4 hours, of the event. After certain primarily parenteral exposures to inciting agents such as drugs or stinging insects, the severity of anaphylaxis has been shown to positively correlate with the degree to which the total tryptase level increases over baseline.¹⁷ These clinical findings have led this defined increase (20+2) over BST to be cited as a criterion for the diagnosis of mast cell activation syndrome.¹⁸ Indeed, studies have supported the sensitivity of such an approach after parenteral exposures,^{19,20} and currently, 20+2 is held as the gold standard for confirmation of mast cell activation in all clinical settings.¹⁶ However, IgE-mediated immediate hypersensitivity reactions to other antigens, such as ingestion of foods in allergic

individuals, have been shown to result in changes in total tryptase levels that may frequently fail to meet the 20+2 threshold despite resulting in severe reactions in some cases.²¹⁻²⁴ In addition to the route of exposure, this limitation in sensitivity may result in part from the observation that, in particular, young individuals with food allergy often have low BST levels.²² Conversely, it had been speculated that a high BST might negatively impact the specificity of the 20+2 algorithm.^{15,25,26}

To address this potential concern, Mateja et al²⁵ set out to define baseline variability in BST levels, an aspect of this biomarker that had not previously been characterized. The study found that BST levels were similarly labile among allergic individuals with normal BST levels (median 4.1 ng/mL), as well as individuals with elevated levels caused by SM, H α T, or both (median 33.6 ng/mL). In some cases, BST levels doubled between measurements, regardless of the baseline level. Owing to the higher BST levels seen in patients with SM and/or H α T, baseline variability thus negatively impacted the specificity of the 20+2 algorithm. Among individuals with elevated BST due to SM and/or H α T, 24% (33 of 139) of serial measurements would have met the 20+2 threshold, even though none of the individuals were reported to be experiencing acute symptoms of immediate hypersensitivity (Figure 1). Moreover, the measurement of mature tryptases in serum did not contribute to this variability, indicating that these changes were from constitutive secretion of pro-tryptases, and not a result of mast cell degranulation.

Using a statistical model, the authors proposed a new threshold-based approach, where an increase of 68.5% over BST (or 1.685 times BST) was found to both accurately identify anaphylaxis (ie, sensitivity) and correctly classify subjects as not having meaningful increases in tryptase when they were at baseline (ie, specificity).²⁵ Thus, at this threshold, sensitivity and specificity were jointly optimized (ie, the sum of sensitivity and specificity was maximized). Using the new optimized threshold, only 4.3% (6 of 139) of individuals with elevated BST in the study would have been misidentified as having a clinically meaningful increase in serum tryptase that would be suggestive of anaphylaxis (Figure 1). Both algorithms performed similarly for confirmation of known anaphylaxis cases (ie, were similarly sensitive) and are identical for individuals who have the median tryptase level (4.1 ng/mL) for individuals where H α T, renal disease, and clonal myeloid neoplasms including SM had been excluded.

In addition to the optimized threshold, the authors offered 2 additional thresholds that could be used based on a pretest judgment that the reaction may or may not have been a mast cell-mediated systemic immediate hypersensitivity reaction to maximize sensitivity or specificity, respectively. For a reaction thought unlikely to be anaphylaxis—such as systemic symptoms after passive inhalation of an odor—the authors proposed a high specificity threshold of 1.868, or an 86.8% rise over BST. Conversely, for reactions where anaphylaxis was believed to be likely—such as a systemic reaction after an oral food challenge in a food allergic patient—the authors proposed a high sensitivity threshold of 1.374, or a 37.4% rise over BST (Figure 1). The authors also generated an online application for use in clinical practice: <https://triptase-calculator.niaid.nih.gov/>. Although additional studies are needed to examine the real-world sensitivity and specificity of this new approach in different populations, one recent retrospective study examining causes for

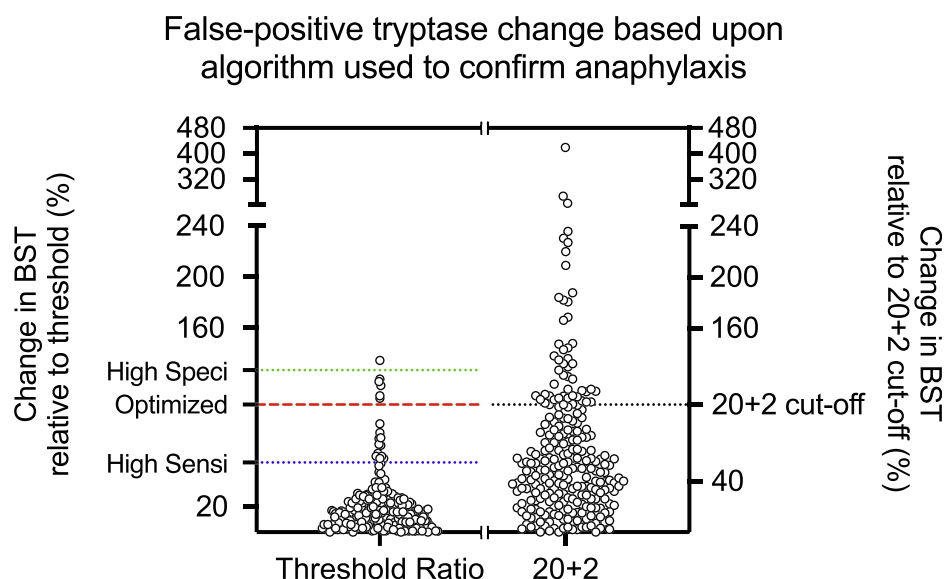


FIGURE 1. Comparison of false-positive rates for serial basal serum tryptase (BST) measurements in patients with systemic mastocytosis and hereditary α -tryptasemia using the threshold ratio approach versus the 20% + 2 ng/mL algorithm (20+2). Individual values for sequential BST measurements are shown as a percentage of the respective algorithm. The 20+2 algorithm and cutoff (black dotted line) are shown on the right, whereas threshold ratios are shown on the left for the same individuals. High sensitivity (Sensi) threshold ratio indicated by bottom (blue dotted line), high specificity (Speci) threshold ratio indicated by the top (green dotted line), and the threshold ratio optimized to maximize both sensitivity and specificity (Optimized) indicated by the middle (red dashed line). Adapted from O'Connell and Lyons.²⁶

elevated BST in a regional health system also found that BST levels were labile.²⁷ In this study, 41.5% ($n = 22$ of 53) of those with elevated BST due to any cause would have met the 20+2 threshold, whereas only 7.5% ($n = 4$ of 53) would have met the optimized threshold at baseline. Future studies examining the sensitivity of this proposed approach, including among individuals with food allergic reactions and very low or extremely high BST levels, are needed and are currently lacking.

H α T: THE COMMON CAUSE OF ELEVATED BST

An estimated 6% of people in the general population have elevated BST levels of 11.4 ng/mL or higher.⁹ However, multiple studies from the United States, United Kingdom, and European Union have now demonstrated that H α T is comparably prevalent, also affecting approximately 6% of the general populations studied (Figure 2, gray bars).²⁸⁻³¹ Although H α T has rarely been reported among individuals with BST as low as 6.5 ng/mL, the median level for individuals with a single *TPSAB1* replication is 13.6 ng/mL (95% confidence interval [CI]: 12.6-14.4 ng/mL).¹⁵ Higher order copy number is associated with even higher BST levels, where 2 additional *TPSAB1* copies result in BST levels of 22.5 ng/mL (95% CI: 19.4-23.6 ng/mL), and in the 1 family reported with 4 extra germline *TPSAB1* copies, BST levels were 37 ng/mL (95% CI: 25.5-62.7 ng/mL).³² Up to 10 additional *TPSAB1* levels have now been reported with BST levels averaging 133 ng/mL (95% CI: 110-156 ng/mL) in the 2 identified individuals (Table 1).³³ Although copy number variation in β -tryptase encoding sequences has also been reported,^{28,31,34-36} increased β -tryptase encoding gene copy number has yet to be associated with inherited elevations in BST.³⁷ This appears to be a result of a coinherited regulatory elements in replicated *TPSAB1* gene

promoters that lead to overexpression of the duplicated locus.³³ So far, the available data also indicate that *TPSAB1* replications encode wild-type α -tryptase sequences. Thus, H α T may be thought of as a natural overexpression model of α -tryptase.

Based on the combined prevalence data of H α T and elevated BST in the general population, as well as those available for the prevalence of advanced renal disease and clonal myeloid disorders,³⁸ estimates have been made about the common causes for elevated BST when encountered clinically.⁹ H α T would be the most common cause for elevated BST, accounting for estimated approximately 91% of individuals with levels >11.4 ng/mL (Figure 3). Although other genetic conditions such as GATA-binding protein 2 (GATA2) haploinsufficiency³⁹ and Gaucher's disease⁴⁰ have anecdotally been reported in association with elevated BST levels, these rare conditions and findings are expected to account for less than 1% of individuals with elevated BST. Although acquired clonal myeloid neoplasms are frequently associated with elevated BST levels,^{1,2,14} these diseases, even in aggregate, are also rare and likely only account for approximately 1% of individuals with elevated BST. Of the acquired conditions associated with clinically meaningful increases in BST, advanced chronic kidney disease (ACKD)⁴¹ and end-stage renal disease⁴² are the only relatively common conditions and are predicted to account for approximately 7% of individuals with elevated BST (Figure 3).

PREVALENCE AND CONSEQUENCE OF H α T IN PATIENTS WITH SM

In 2018, Sabato et al³² reported the first family identified with a *TPSAB1* quintuplication encoding α -tryptase. In one affected family member, clonal mast cell disease was also found. The

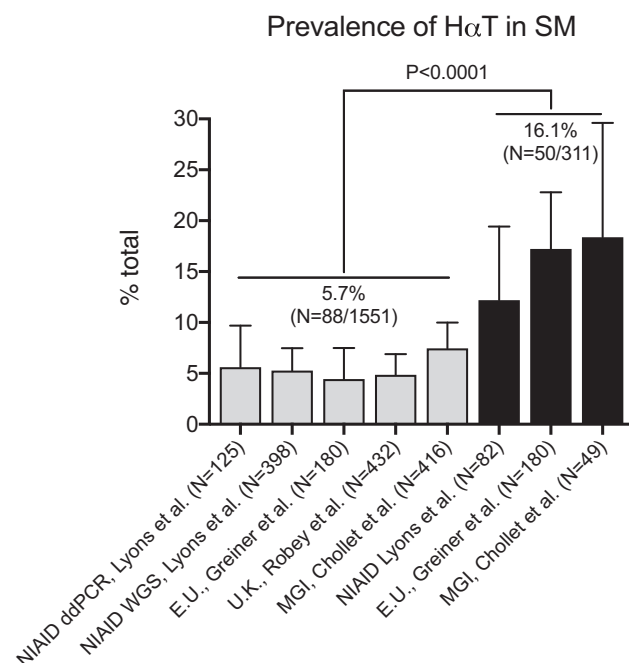


FIGURE 2. Prevalence of hereditary α -tryptasemia (H α T) among individuals with systemic mastocytosis (SM) compared with controls from the United States, United Kingdom (U.K.), and European Union (E.U.). Prevalence data among healthy individuals or biorepositories from 4 independent studies²²⁻²⁵ and among patients with SM from 3 independent studies.²²⁻²⁴ Light gray bars indicate control population prevalence data, and black bars indicate prevalence data from SM cohorts. *ddPCR*, Droplet digital polymerase chain reaction; *MGI*, Michigan Genomics Initiative; *NIAID*, National Institute of Allergy and Infectious Diseases; *WGS*, whole genome sequencing. Adapted from O’Connell and Lyons.²⁶

authors noted at the time that it was curious to have 2 presumably rare conditions occurring in the same family; indeed this remains the only kindred with 4 *TPSAB1* replications reported to date, and the prevalence of SM is estimated at 1 in 10,000.^{43,44} On the basis of this, the authors suggested that perhaps this association was not happenstance, but rather a harbinger of a link between clonal mast cell disease and H α T.

Since this original observation, there have been 3 studies which have demonstrated a remarkable association between H α T and SM, where H α T was present in 12% to 18% of the cohorts examined in the United States and European Union²⁸⁻³⁰—a level 2 to 3 times that of the general population (Figure 2, black bars). Patients with SM and concomitant H α T were found to be twice as likely to present with a history of systemic anaphylaxis compared with those without H α T in the 2 largest studies to examine this association.^{29,30} Moreover, in the largest study of patients with SM published as of this manuscript, a gene-dosage effect was reported whereby an increase in the number of *TPSAB1* copies encoding α -tryptase was associated with more severe mast cell mediator symptoms as measured using a validated symptom assessment score.²⁹ A subsequent study was not able to confirm this result using unvalidated self-reported measures, though the study design was not powered to detect a difference based on the small sample size.²⁸ However, the increased prevalence of H α T shown in all 3 studies strongly

TABLE I. BST levels and predicted upper limits dictated by additional *TPSAB1* copy encoding α -tryptase defined by genotype

Additional <i>TPSAB1</i> copy number	0	1	2	3	4	6	10
Tryptase genotype* (<i>TPSAB1</i> , <i>TPSB2</i>)	$\beta, \beta/\beta, \beta; \alpha, \beta/\alpha, \beta; \alpha, \beta/\beta, \beta, \beta, \beta; \beta, \beta/\beta, \beta, \beta; \beta/\alpha, \beta; \beta/\beta, \beta$	$\alpha\alpha, \beta/\beta, \beta; \alpha\alpha, \beta/\alpha, \beta; \alpha\alpha, \beta/\beta, \beta, \beta, \beta; \alpha\alpha, \beta/\alpha, \beta$	$\alpha\alpha, \beta/\alpha, \beta; \alpha\alpha, \beta/\beta, \beta, \beta, \beta; \alpha\alpha, \beta/\alpha, \beta$	$\alpha\alpha\alpha, \beta/\alpha\alpha, \beta$	$\alpha\alpha\alpha\alpha, \beta/\beta, \beta; \alpha\alpha\alpha\alpha, \beta/\alpha, \beta$	$\alpha\alpha\alpha\alpha, \beta/\alpha\alpha, \beta$	$\alpha\alpha\alpha\alpha\alpha\alpha\alpha\alpha, \beta/\alpha, \beta$
BST (ng/mL), median (range)	4.1 (0-10.4)	13.6 (6.5-33.9)	22.5 (10.5-39.5)	27.3 (23.4-40)	37 (25.5-62.7)	87 (NA)	133 (110-156)
Upper 99.5% PI for BST (ng/mL)	11.4	36.2	62.2	88.8	115.9	171.2	285.1
Upper limit using BST/(1+copy) [†]	20	40	60	80	100	140	220

BBST, Basal serum tryptase; *NA*, not applicable; *PI*, prediction interval; *TPSAB1*, tryptase α/β -1; *TPSB2*, tryptase β -2.

*Trypsin genotypes identified to date for this number of *TPSAB* / replications. In all cases, *TPSAB* / replications associated with elevated BST identified encode α -tryptase.

*Upper limits were generated by inverting this formula such that the threshold was the current minor criterion of 20 ng/mL times one plus the replication number.

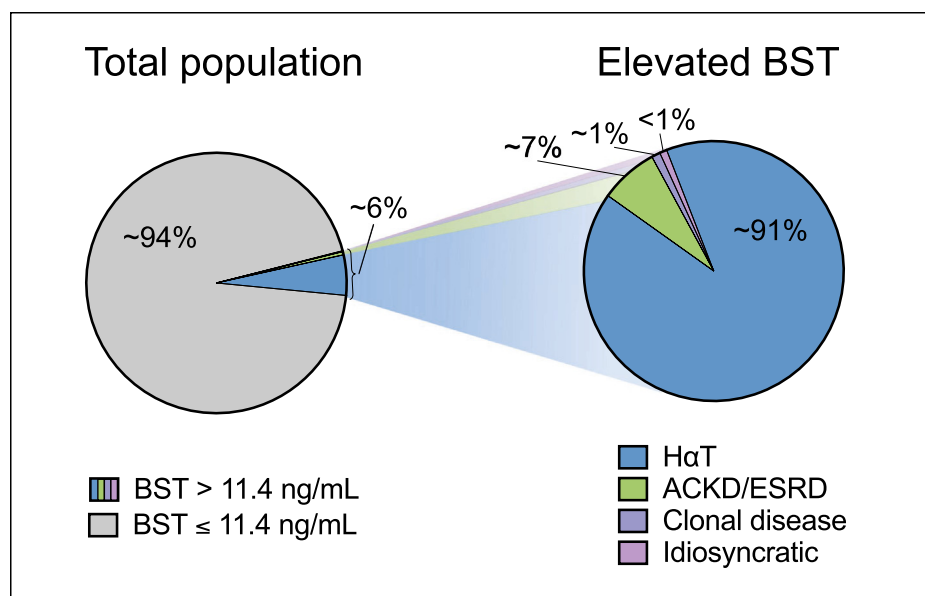


FIGURE 3. Hereditary α -tryptasemia (H α T) is the common cause of elevated basal serum tryptase (BST) levels in the general population. The estimated prevalence of elevated BST and associated causes in Western populations. Clonal disease includes mastocytosis and other myeloid neoplasms including acute and chronic myeloid leukemias, myelodysplastic syndromes, myeloproliferative variant hyper-eosinophilic syndrome, and related disorders. Idiosyncratic cases include rare inherited causes such as families with pathogenic *GATA2* or *PLCG2* variants, as well as acquired causes including but not limited to certain helminth infections. *ACKD*, Advanced chronic kidney disease; *ESRD*, end-stage renal disease. Adapted from Lyons.⁹

supports the hypothesis that H α T modifies clinical phenotypes associated with SM—in particular, immediate hypersensitivity symptoms. Although it remains possible—given that an increased number of mast cells have been reported in the bone marrow and gastrointestinal mucosae of symptomatic individuals with H α T^{7,45–47}—that this genetic trait could promote the development of SM, it currently seems more likely that H α T results in more significant mast cell–associated symptoms such that patients with both H α T and mastocytosis more often require medical attention.

Although the mechanism(s) underlying the modification of immediate hypersensitivity and mast cell mediator–associated symptoms by H α T remain incompletely understood, α / β -tryptase heterotetramers have been posited as a potentially contributing factor.^{30,48} Mature, enzymatically active β -tryptase tetramers have trypsin-like serine proteinase activity.⁴⁹ However, α -tryptase tetramers—due to variants that block the access of peptide substrates to the enzyme active site—lack *in vitro* enzymatic activity.^{50,51} Despite this finding, it was noted several years ago that mature α -tryptase tetramers were much more stable molecules and, unlike β -tryptase tetramers, did not require heparin to form stable mature enzyme.⁵¹ Heterotetrameric mature tryptases were only recently characterized and seem to exhibit both the stability imparted by α -tryptase subunits and the enzymatic activity of the β -tryptase subunits, yielding mature enzyme that is more stable at neutral pH and exhibits an expanded substrate specificity.⁴⁸ Among the targets shown to be selectively cleaved by α / β -tryptase heterotetramers are the protease activated receptor-2 (PAR-2) and the EGF-like module-containing mucin-like hormone receptor-like 2

(EMR2) that are associated with vascular epithelial cell leak and vibration-induced mast cell activation *in vitro*, respectively. Although no drug or biologic is currently available to block tryptase activity, both small molecules and monoclonal antibodies have been or are currently in clinical development.^{35,52}

INCORPORATION OF TRYPTASE GENOTYPING IN THE EVALUATION OF PATIENTS WITH SUSPECTED CLONAL MYELOID NEOPLASMS INCLUDING MASTOCYTOSIS

Because H α T accounts for most individuals with elevated BST, integrating tryptase genotyping into the workup of patients suspected of having a mast cell–associated disorder or a myeloid neoplasm has been shown to improve identification of clonal myeloid disorders.³³

The human tryptase locus on chromosome 16 at position p13.3 contains 4 paralogous genes *TPSG1*, *TPSB2*, *TPSAB1*, and *TPSD1*.¹² *TPSG1* encoding γ -tryptase is thought to be the first to evolve in humans and contains a transmembrane domain preventing it from being secreted.⁵³ The other 3 tryptase genes encode for secreted tryptase proteins. However, δ -tryptase encoded by *TPSD1* lacks a C-terminal sequence important for enzyme specificity and has been predicted to be enzymatically inactive,⁵⁴ though recombinantly expressed protein has shown some enzymatic activity *in vitro*.⁵⁵ Very little is known about the biological relevance or function of either γ - or δ -tryptases.

It is believed that the biologically relevant secreted tryptases are α - and β -tryptases. Whereas either isoform may be encoded at *TPSAB1*, only β -tryptases are known to be encoded at *TPSB2*.

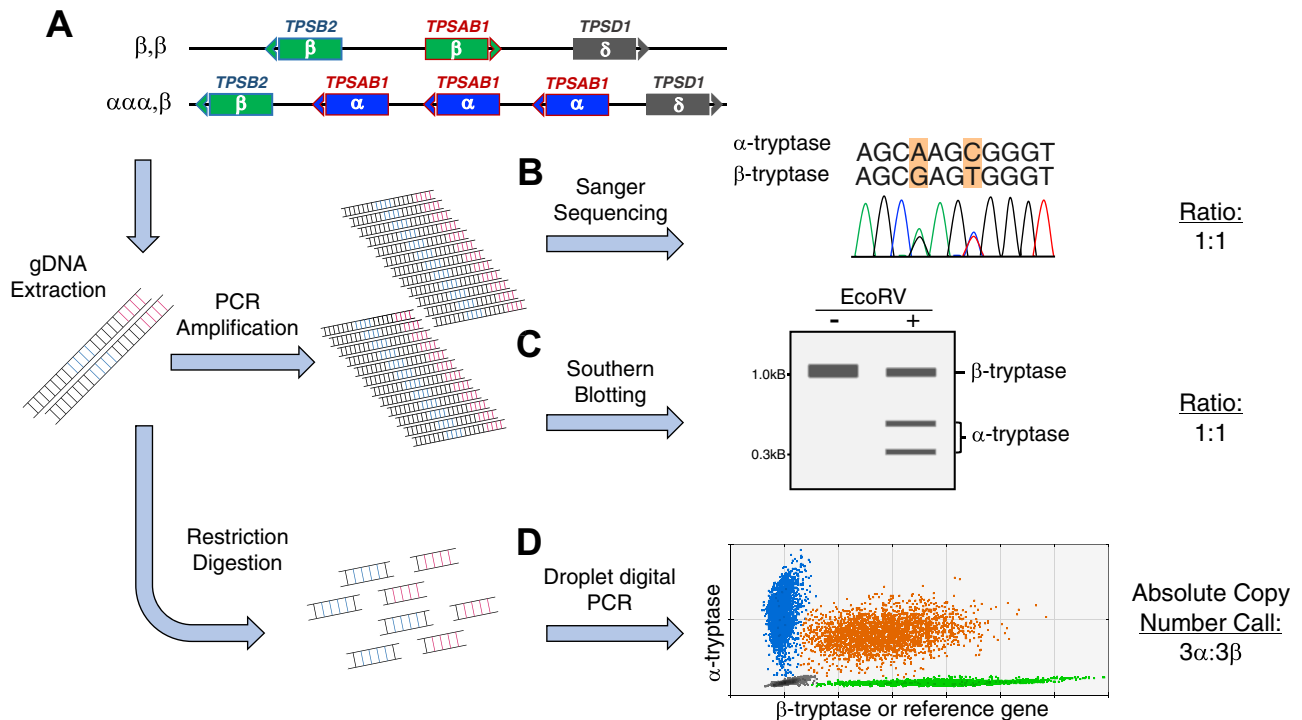


FIGURE 4. Tryptase genotyping strategies. (A) Two alleles in an individual with hereditary α -tryptasemia (H α T)—one allele with 2 β -tryptase sequences—one each at *TPSAB1* and *TPSB2* (top), and the second H α T-associated allele with 3 α -tryptase encoding *TPSAB1* copies and a single β -tryptase encoding *TPSB2* copy (bottom). Genomic DNA (gDNA) extracted from cells from this individual, containing these 6 α - or β -tryptase sequences, is either amplified or restriction digested. The amplified gDNA can then either be (B) Sanger sequenced or (C) treated with the restriction enzyme *EcoRV* and Southern blotted to perform relative quantitation of α - and β -tryptase sequences. In both cases, the ratio of calculated α - to β -tryptase sequences would be 1:1. (D) Unamplified digested gDNA is assayed by droplet digital PCR (ddPCR), which allows for absolute copy number detection of α - and β -tryptase sequences, yielding the genotype determination 3 α :3 β . PCR, Polymerase chain reaction; *TPSAB1*, tryptase α/β -1; *TPSB2*, tryptase β -2; *TPSD1*, tryptase δ -1. Adapted from Lyons.⁵⁶

Because of a high degree of sequence homology between α - and β -tryptases and repetitive sequences flanking both genes, traditional methods of copy number determination such as microarray-based comparative genomic hybridization (aCGH) or other clinically available next-generation sequencing technologies such as whole exome or genome sequencing (WES or WGS), respectively, cannot accurately determine the number of α - and β -tryptase encoding copies.

To overcome these issues, a droplet digital polymerase chain reaction (ddPCR)-based assay was developed for clinical use (Figure 4).⁵⁶ Unlike previous research and most clinically available assays, that require amplification of genomic DNA to sequence or quantify differences in copy number; ddPCR directly measures the quantity of a given DNA sequence relative to the quantity of a reference gene with conserved copy number (2n) in the same reaction and can accurately determine tryptase genotypes. Although this assay currently has limited availability, we anticipate that the recent adoption of an International Classification of Diseases, Tenth Revision, Clinical Modification (ICD-10-CM), code for H α T (D89.44), as well as expanded use at referral centers, and the demonstrable utility of incorporating tryptase genotyping into the workup of myeloid neoplasms and diagnostic algorithm for SM will lead to expanded availability of this assay.

Several publications have proposed algorithms in which tryptase genotyping may be incorporated into the workup of

patients suspected of having a clonal myeloid neoplasm or mast cell—associated disorder.^{11,27,38} Although there remains no consensus proposal for how to incorporate tryptase genotyping into the workup of clonal myeloid and mast cell neoplasms, many referral centers are already doing so. An example of such an algorithm is included here (Figure 5). As this technology and testing becomes more widely available clinically, we anticipate tryptase genotyping becoming an integral step in the evaluation of patients with elevated BST. In a retrospective study of a regional health system by Waters et al²⁷ where such an approach was applied, 93% (54 of 58) of individuals with BST >11.4 ng/mL had H α T, a myeloid neoplasm, or ACKD. In a second larger study by Chovanec et al,³³ it was demonstrated that more than 98% (304 of 309) of patients referred with BST >11.4 ng/mL had SM, H α T, or both. In this second study, the authors went on to sequence bone marrow aspirate samples from the 5 patients with BST >11.4 ng/mL who did not have either SM or H α T. One patient met criteria for idiopathic hypereosinophilic syndrome, and in all 5 individuals, somatic (n = 4) or germline (n = 1) variants were identified to be suggestive of evolving clonal hematopoiesis of indeterminate potential in the myeloid compartment. On the basis of these findings, the authors concluded that when a BST level is above 11.4 ng/mL in the absence of H α T or advanced renal disease, myeloid neoplasia—including SM—is the presumed diagnosis and must be excluded.

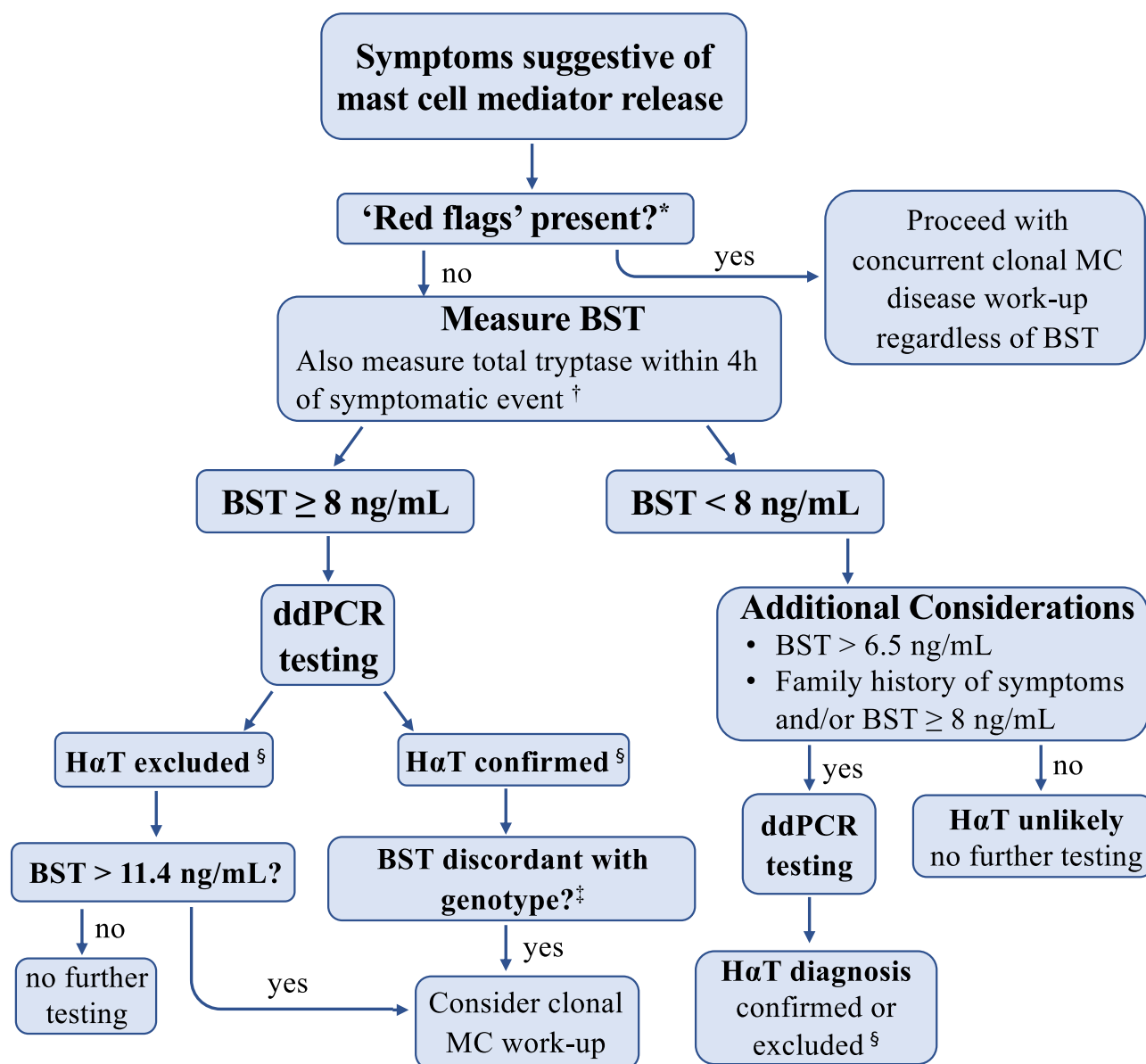


FIGURE 5. Clinical workup of patients with elevated basal serum tryptase (BST) incorporating tryptase genotyping. *“Red flags” may include, but are not limited to hepatosplenomegaly, lymphadenopathy, CBC abnormalities (eg, thrombocytopenia, anemia, polycythemia, neutrophilia, eosinophilia [AEC >1500 cells/mL]), severe and/or recurrent anaphylaxis (in particular, idiopathic or Hymenoptera venom-induced), urticaria pigmentosa/Darier’s sign, eosinophilic tissue infiltration, premature osteopenia/osteoporosis, or pathological fracture; †24-hour urinary N-methylhistamine and/or 2,3-dinor-11β PGF2α can also be sent where available; §HαT is diagnosed by the presence of increased α-tryptase encoding *TPSAB1* copy number; ‡See Table 1 for predicted upper 99.5% CI of the BST level based on *TPSAB1* replication. AEC, Absolute eosinophil count; CBC, complete blood count; CI, confidence interval; ddPCR, droplet digital polymerase chain reaction; HαT, hereditary α-trypsinemia; MC, mast cell. Adapted from Luskin et al.³⁸

TRYPTASE GENOTYPES DEFINE REFERENCE RANGES FOR BST AND IMPACT THE DIAGNOSTIC CRITERIA FOR SM

The current upper limit of normal for BST in most clinical laboratories is currently 11.4 ng/mL. However, this cutoff is based solely on pseudonormalization of BST values that are non-normally distributed, and generally a cutoff of 20 ng/mL or more is suggestive of a clonal mast cell or myeloid neoplasm. Given the observation that the substantial majority of individuals with

“elevated” BST could be accounted for by 1 of 3 diagnoses (ie, HαT, renal failure, or myeloid neoplasm), Chovanec et al³³ set out to define what would be considered a “normal” BST based on data from healthy volunteers and family members of patients where tryptase genotype, clinical history, and blood work would exclude all 3 diagnoses. Remarkably, they found the upper limit of the 99.5% prediction interval (PI) to be 11.4 ng/mL—likely due in part to the relative prevalence of HαT and standard exclusion of approximately 5% of the outliers (eg, 95% CI) in

TABLE II. Signs and symptoms suggestive of occult clonal myeloid or mast cell disease

- Lymphadenopathy
- Hepatosplenomegaly
- CBC abnormalities
 - thrombocytopenia
 - anemia
 - pancytopenia
 - polycythemia
 - neutrophilia
 - hypereosinophilia (AEC >1500 cells/ μ L)
- Eosinophilic tissue infiltration and/or inflammation
- Anaphylaxis
 - idiopathic
 - venom
 - severe reactions with syncope and/or hemodynamic instability
- Urticaria pigmentosa/Darier's sign
- Premature osteopenia/osteoporosis or pathological fracture
- BST discordant with *TPSAB1* copy number*

AEC, Absolute eosinophil count; BST, basal serum tryptase; CBC, complete blood count; *TPSAB1*, tryptase α/β -1.

*Each additional *TPSAB1* copy number encoding α -tryptase increases BST by approximately 9 ng/mL (see Table I).

generating standard curves from non-normal data. Thus, the authors suggested that in the absence of H α T, a BST >11.4 ng/mL be considered abnormal and proposed that it may be used as a minor criterion for SM in this context.

They also modeled BST levels among individuals with H α T based on their data showing each *TPSAB1* replication encoding α -tryptase appears to be similarly overexpressed, leading to a linear gene-dosage effect on BST levels (Table I). In doing so, the authors generated prediction intervals (PIs) based on α -tryptase encoding *TPSAB1* replication number, above which they recommended that an investigation for a concomitant myeloid neoplasm be considered. These cutoffs were incorporated into an online application intended for clinical use (<https://bst-calculator.niaid.nih.gov/>), and it was suggested that these cutoffs may be used, where genotyping is available, as minor criteria for SM when H α T is also present, instead of the current consensus cutoff of 20 ng/mL. An alternative method of adjusting for H α T had been previously suggested as well, where patient BST levels could be divided by 1 plus the total number of extra *TPSAB1* copies (BST/(1+copy)) (Table I), and then the current minor criterion of 20 ng/mL would still be used.⁵⁷ Although less precise, this approach approximates the data-driven model, where the upper 99.5% PI for a *TPSAB1* duplication (the most common H α T genotype) is 36.2 ng/mL ($36.2/(1+1) = 18.1$), the 99.5% PI for a *TPSAB1* triplication is 62.2 ng/mL ($62.2/(1+2) = 20.7$), and so on.

Whether either model might impact interpretation of the current B-Finding BST ≥ 200 ng/mL in the evaluation of patients with SM is currently unclear; no corrections have been proposed. Because each *TPSAB1* replication encoding α -tryptase adds approximately 10 ng/mL, a BST level of 200 ng/mL due to H α T, while not impossible, would be predicted to result from approximately 20 *TPSAB1* replications. Because one individual has been identified with 10 replications, this situation is not an impossibility (eg, 2 individuals with 10 replications each, could have offspring that inherit 10 replications from both parents). One correction possibility might be to subtract the predicted

median BST for a given genotype from the total BST based on the data-driven BST CALCULATOR (<https://bst-calculator.niaid.nih.gov/>), while retaining the 200 ng/mL cutoff for a B-Finding. How the BST/(1+copy) method might be used to correct BST in this context is unclear.

Overall, the data-driven approach employing the online calculator appears to offer an advantage over the BST/(1+copy) method as it not only enables a more precise BST correction for *TPSAB1* replication number but would also correct the minor BST criterion for SM in patients without H α T (to 11.4 ng/mL from 20 ng/mL). Whether either approach will be adopted in consensus recommendations remains to be determined, and additional studies are needed to validate these proposed cutoffs. Indeed, 1 individual in the H α T modeling dataset fell outside of the PI for unclear reasons, but this was potentially due to a number of comorbidities the study cited. What remains clear is that employing the use of BST measurements along with reflex tryptase genotyping of patients with BST >8 ng/mL (and in some selected cases >6.5 ng/mL) (Figure 5) as well as routine screening for *KIT* p.D816V in peripheral blood of patients with phenotypes strongly suggestive of clonal mast cell disease irrespective of the BST level³⁸ are highly effective noninvasive means of risk stratifying patients suspected of having mast cell–associated disorders. Moreover, confirming the presence of H α T—with a genotype concordant with the BST level (Table I)—among patients with elevated BST in whom there are no signs or symptoms that would otherwise prompt a more in-depth clinical workup for a myeloid neoplasm (Table II) is likely to preclude a number of unnecessary bone marrow biopsies in the future.

SUMMARY AND FUTURE DIRECTIONS

Advances in the understanding of tryptase genetics, in particular the identification and characterization of H α T and how this genetic trait accounts for most individuals with elevated BST, have fundamentally changed the clinical interpretation of this biomarker. As these data gain more widespread recognition, they will certainly have an impact on future clinical practice guidelines and recommendations for the workup of patients with mast cell–associated disorders and reactions, including SM. Despite the knowledge gained to date, much additional scientific work remains. In addition to mechanistic studies that are needed to better understand the phenotypic impacts of differential tryptase gene expression, additional basic genetic studies enabled by relatively recent advances in technologies are needed to better characterize genetic variability at the human tryptase locus with even greater resolution. As tryptase genetic variability, composition, and the impacts of these on clinical phenotypes continue to be better defined, we envision that this knowledge will be incorporated not only into clinical practice but also anticipate an expanded role in clinical trial design for diseases associated with mast cells in the future.

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