Interpreting Tryptase Levels and Avoiding Common Pitfalls in Screening for Clonal Mast Cell Disease

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Background

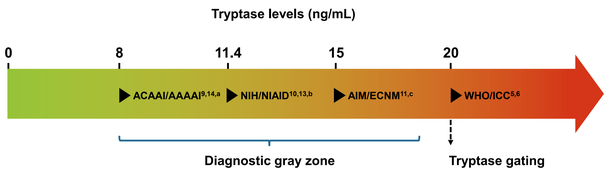
- Clonal mast cell diseases (cMCD) are driven mainly by the *KIT* D816V mutation; about 95% of patients with systemic mastocytosis (SM), the most common cMCD subtype, harbor this variant[1-5]
- According to the World Health Organization (2022) and International Consensus Classification (2022), SM diagnostic criteria include presence of mast cell (MC) aggregates in noncutaneous tissues, alongside four minor criteria, which include basal serum tryptase (BST) levels of >20 ng/mL in peripheral blood (PB)[5-7] (**Table 1**, **Figure 1**)
- Identifying patients with cMCD can be a diagnostic challenge due to nonspecific, variable symptoms and an evolving understanding of what constitutes a "normal" tryptase[2,4,5,8-11]
- Incorrect use of the diagnostic threshold of BST >20 ng/mL as a screening threshold to rule out cMCD (that is, "tryptase gating") can lead to missed or delayed diagnoses[12]
- Recent guidance from the American College of Allergy, Asthma, and Immunology recommends that BST >8 ng/mL should be considered suggestive of cMCD or hereditary alpha-tryptasemia (HaT, a genetic trait that leads to elevated BST)[9] (**Figure 1**)
- PROSPECTOR (NCT04811365) was a multicenter, prospective screening study that evaluated prevalence of the *KIT* D816V mutation in PB of patients with anaphylaxis or symptoms consistent with systemic MC activation[4]
  - BST was also collected and measured via central lab testing in PROSPECTOR, allowing for *post hoc* examination of BST levels in this cohort of patients with cMCD
- In this exploratory analysis, we aimed to evaluate the diagnostic utility and reliability of BST levels in predicting cMCD in a subset of patients without HaT from the PROSPECTOR screening study[4]

Table 1. WHO (2022)/ICC (2022) diagnostic criteria for systemic mastocytosis[5,6,9]

Major criteria
Multifocal aggregates of mast cells (15 or more per aggregate) in a tissue biopsy other than skin
Minor criteria
Morphologically abnormal mast cells comprising 25% or more of the infiltrate such as spindle-shaped mast cells in bone marrow biopsy and aspirates or other tissues
Aberrant expression of CD25, CD2 and/or CD30 by mast cells (can be detected by flow cytometry or immunohistochemistry)
Presence of *KIT* D816V or another activating *KIT* mutation in lesional tissue or peripheral blood
BST level of >20 ng/mL (does not apply in patients with associated hematologic disorders)[a]

[a]WHO recommends adjusting BST levels for patients with HaT.
BST, basal serum tryptase; CD, cluster of differentiation; HaT, hereditary alpha-tryptasemia; ICC, International Consensus Classification; WHO, World Health Organization.

Figure 1. Guideline recommendations for BST threshold in cMCD

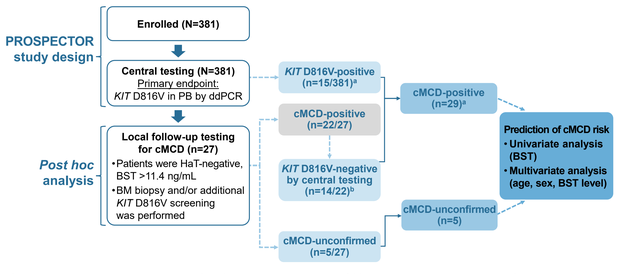


[a]HaT or mastocytosis should be suspected in patients with BST above 8 ng/mL. However, when BST is above 11.4 ng/mL and HaT is absent, cMCD or another myeloid neoplasm should be suspected. [b]When BST is above 11.4 ng/mL and HaT is absent, cMCD may be frequently identified. [c]Symptomatic patients with BST above 15 ng/mL should be screened for HaT and *KIT* D816V mutation.
AAAAI, American Academy of Allergy, Asthma, and Immunology; ACAAI, American College of Allergy, Asthma, and Immunology; AIM, American Initiative in Mast Cell Diseases; BST, basal serum tryptase; cMCD, clonal mast cell diseases; ECNM, European Competence Network on Mastocytosis; HaT, hereditary alpha-tryptasemia; ICC, International Consensus Classification; NIAID, National Institute of Allergy and Infectious Diseases; NIH, National Institutes of Health; WHO, World Health Organization.

Methods

- PROSPECTOR (NCT04811365; N=381) was a multicenter, prospective screening study that evaluated prevalence of the *KIT* D816V mutation in PB by central laboratory testing using droplet digital PCR (ddPCR, limit of detection ≥0.03%) in adult patients with anaphylaxis or symptoms consistent with systemic MC activation (**Figure 2**)[4]
- Patients met enrollment criteria in ≥1 enrollment groups[4]
  - **Hymenoptera group:** moderate-to-severe anaphylaxis (Ring-Messmer grade ≥II) due to Hymenoptera sting
  - **20% + 2 tryptase group:** moderate-to-severe anaphylaxis (Ring-Messmer grade ≥II) with cardiovascular involvement and documented event-related tryptase elevation fitting the formula 20% of baseline plus 2 ng/mL evaluated in ≥1 event
  - **Cardiovascular group:** BST levels ≥8 ng/mL, cardiovascular involvement (tachycardia, syncope, and hypotension), and involvement of ≥1 other organ system, characterized by skin (pruritus, urticaria, flushing, and angioedema), gastrointestinal (diarrhea, nausea, vomiting, and gastrointestinal cramping), or respiratory/naso-ocular symptoms (wheezing, conjunctival injection, and nasal stuffiness)
- cMCD was defined as patients without HaT who were either
  - *KIT* D816V-positive in PB by central testing or
  - Diagnosed by investigators through *post hoc* local follow-up evaluations in patients with elevated BST (>11.4 ng/mL) who were previously negative for *KIT* D816V in central testing (**Figure 2**)
- Distribution of patients diagnosed with cMCD in central testing and follow-up studies (n=28) were assessed across BST strata
- Univariate logistic regression was performed to evaluate BST as the sole predictor of cMCD
- A multivariable exploratory model included age, sex, and BST as predictors of cMCD risk

Figure 2. PROSPECTOR study design, central testing, and *post hoc* evaluation



[a]One patient was HaT-positive and excluded from further analysis in this study. [b]Of the 22 patients diagnosed with cMCD in the local follow-up testing, 14 were negative for *KIT* D816V by previous central PB testing and included in this study.
BM, bone marrow; BST, basal serum tryptase; cMCD, clonal mast cell disease; ddPCR, droplet digital polymerase chain reaction; HaT, hereditary alpha-tryptasemia; PB, peripheral blood.

Results

- In PROSPECTOR, 29/381 (8%) patients were diagnosed with cMCD
- Central PB testing revealed 15/381 patients with detectable *KIT* D816V; 1/15 patients was also diagnosed with HaT
- Local assessment in 27 patients with elevated BST (>11.4 ng/mL) and no HaT led to cMCD diagnosis in 22 (81%) patients; 14/22 (64%) were negative for *KIT* D816V by previous central PB testing
  - In the remaining 5/27 patients, cMCD was not confirmed due to absence of bone marrow biopsy
- Altogether, 33 patients were assessed in this study: 28 patients with cMCD and no HaT, and 5 patients with unconfirmed cMCD (**Table 2**)
  - Mean age was 54 years, and 15/33 (45%) were female
  - Of 33 patients, 27 (82%) had BST <20 ng/mL; *KIT* D816V was detectable in 25/33 (76%) patients
  - Severe anaphylaxis following Hymenoptera sting represented the most frequent basis for study inclusion, with 21/33 patients (64%) meeting this criterion. Cardiovascular involvement accounted for 18 patients (55%), while 11 patients (33%) fulfilled the 20% + 2 tryptase criterion

Results

Table 2. Demographics and clinical characteristics of PROSPECTOR participants in the study

Parameters	Total (N=33)	Patients with *KIT* D816V by central testing (n=14)[a]	Patients with follow-up (n=27)[b]	
			Confirmed cMCD (n=14)	No confirmed cMCD (n=5)
Age, years				
Mean (SD)	54 (13.79)	54 (13.22)	54 (13.61)	56 (18.56)
Median (min, max)	57 (27, 78)	58 (27, 72)	56 (31, 75)	57 (28, 78)
Female, n (%)	15 (45)	9 (64)	3 (21)	3 (60)
Race, n (%)				
Black or African American	1 (3)	1 (7)	0	0
White	17 (52)	8 (57)	6 (43)	3 (60)
Other	1 (3)	0	0	1 (20)
Unknown	1 (3)	0	1 (7)	0
Not reported	13 (39)	5 (36)	7 (50)	1 (20)
Distribution by inclusion criteria, n (%)				
Hymenoptera group	21 (64)	8 (57)	10 (71)	3 (60)
20% + 2 tryptase group	11 (33)	6 (43)	5 (36)	0
Cardiovascular group	18 (55)	6 (43)	7 (50)	5 (100)
BST level, ng/mL, n (%)				
≤0.0 – <8.0	1 (3)	1 (7)	0	0
≤8.0 – <11.4	2 (6)	2 (14)	0	0
≤11.4 – <15.0	12 (36)	2 (14)	9 (64)	1 (20)
≤15.0 – <20.0	12 (36)	6 (43)	2 (14)	4 (80)
≥20.0	6 (18)	3 (21)	3 (21)	0
***KIT* D816V status, n (%)**				
Positive	25 (76)	14 (100)	11 (79)	0
Undetected	8 (24)	0	3 (21)	5 (100)

[a]One patient who was *KIT* D816V-positive was also HaT-positive and excluded from this study. [b]Diagnosis confirmed via bone marrow biopsy and/or additional *KIT* D816V screening. All patients had BST >11.4 ng/mL and were HaT-negative.
BST, basal serum tryptase; cMCD, clonal mast cell disease; HaT, hereditary alpha-tryptasemia; max, maximum; min, minimum; SD, standard deviation.

- Descriptive statistics showed similar median BST levels between patients with confirmed and unconfirmed cMCD diagnosis (**Table 3**)
  - One extreme BST outlier (200 ng/mL) was observed in the cMCD group

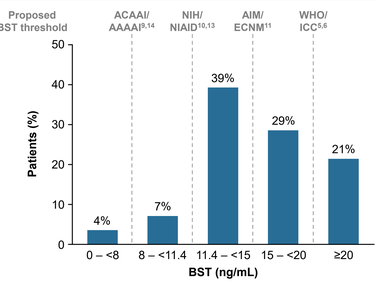
Table 3. BST values among PROSPECTOR participants in the study

BST parameters, ng/mL	cMCD confirmed (N=28)	cMCD not confirmed (N=5)
Mean (SD)	23.2 (35.4)	15.8 (2.6)
Median (min, max)	14.9 (7.2, 200.0)	16.1 (12.0, 19.9)
Interquartile range	12.7 – 19.9	15.6 – 16.3

BST, basal serum tryptase; cMCD, clonal mast cell disease; max, maximum; min, minimum; SD, standard deviation.

- Patients with cMCD were distributed across BST strata (**Figure 3**)
- In the cMCD cohort, 22/28 (79%) patients had BST <20 ng/mL; 19/28 (68%) patients had BST between 11.4 ng/mL and 20 ng/mL (**Figure 3**, **Table 4**)
  - BST was <20 ng/mL in 11/14 (79%) patients positive for *KIT* D816V by central PB testing
  - BST was <20 ng/mL in 11/14 (79%) patients in the *post hoc* follow-up cohort
- For the 5 patients without confirmed cMCD, BST levels were all <20 ng/mL (**Table 3**)

Figure 3. Distribution of PROSPECTOR participants with cMCD (n=28) across BST strata



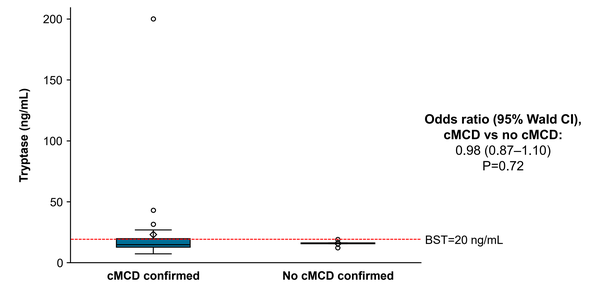
AAAAI, American Academy of Allergy, Asthma, and Immunology; ACAAI, American College of Allergy, Asthma, and Immunology; AIM, American Initiative in Mast Cell Diseases; BST, basal serum tryptase; cMCD, clonal mast cell disease; ECNM, European Competence Network on Mastocytosis; ICC, International Consensus Classification; NIAID, National Institute of Allergy and Infectious Diseases; NIH, National Institutes of Health; WHO, World Health Organization.

Table 4. Proportion of cMCD patients in PROSPECTOR captured by defined BST threshold values

BST threshold	cMCD capture rate
≥8 ng/mL	96%
≥11.4 ng/mL	89%
≥15 ng/mL	50%
≥20 ng/mL	21%

- Multivariable analyses comparing the clonal population (confirmed cMCD diagnosis and no HaT [n=28]) with the control population (unconfirmed cMCD diagnosis due to absence of bone marrow biopsy on follow-up [n=5]) showed no evidence of an association between cMCD and age (odds ratio [95% CI]: 0.9 [0.8–1.1]), sex (9.7 [0.3–340.0]), or BST level (1.0 [0.7–1.4]), although the analysis was underpowered due to small sample size
- In this subset of PROSPECTOR patients, univariate analysis focusing on BST alone revealed no significant association with cMCD, though sample sizes are small (**Figure 4**)

Figure 4. Distribution of BST levels in PROSPECTOR participants with and without cMCD



BST, basal serum tryptase; CI, confidence interval; cMCD, clonal mast cell disease.

Conclusions

- cMCD is frequent in patients with anaphylaxis and can sometimes only be detected by careful assessment of tryptase and *KIT* mutation status
  - In PROSPECTOR, 29 of 381 (8%) patients were diagnosed with cMCD; 25 of 29 (86%) had the *KIT* D816V mutation detected
  - Highly sensitive testing for *KIT* D816V mutation can be helpful in making an early and accurate diagnosis of cMCD
- The majority of patients with underlying cMCD in this pre-selected population with moderate-to-severe anaphylaxis had a BST <20 ng/mL
  - In patients with confirmed cMCD, 22 of 28 (79%) had a BST <20 ng/mL and the median BST was 14.9 ng/mL
- Restricting further work-up for cMCD to patients with a BST greater than 20 ng/mL could miss the majority of cMCD diagnosis, particularly in patients with anaphylaxis
- The role of BST in screening for cMCD and the BST threshold that would be considered normal, needs to be redefined
  - Our results suggest that patients with a BST of >11.4 ng/mL in the absence of HaT should be considered for evaluation of cMCD
  - If HaT testing is not available, a BST threshold of 8 ng/mL as recommended by ACAAI/AAAAI[9,14] would have captured the vast majority (96%) of cMCD patients in our cohort, supporting this value as a highly sensitive threshold to avoid a missed diagnosis of cMCD in patients with anaphylaxis

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