Diagnostic testing approaches for evaluating mastocytosis

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Learning Objectives

- Identify the diagnostic criteria for the clinical diagnosis of mastocytosis
- Interpret biomarkers used to screen for clonal mast cell disease
- Describe how to implement tryptase genotyping
- Recognize that BST is not a good screening tool for clonal mast cell disease in Hymenoptera allergic patients

Diagnosis of Mastocytosis: tissue is (currently) the issue

Table 3.3 WHO diagnostic criteria for systemic mastocytosis[a]

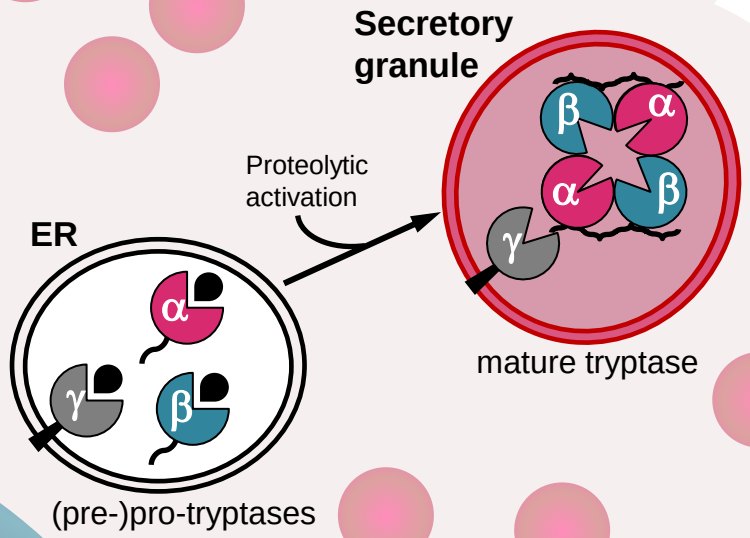
Major criterion
Multifocal dense aggregates of mast cells (≥15/HPF) in bone marrow or extracutaneous sections
Minor criteria
>25% of the mast cells are spindle-shaped, atypical, or immature in morphology
KIT p.D816V or other *KIT* GOF mutation present.
Aberrant expression of CD2 and/or CD25[b] and/or CD30
Total serum tryptase >20 ng/mL[b]

[a]One major and one minor or three minor criteria must be met for diagnosis

[b]Invalid when another clonal myeloid disorder is present

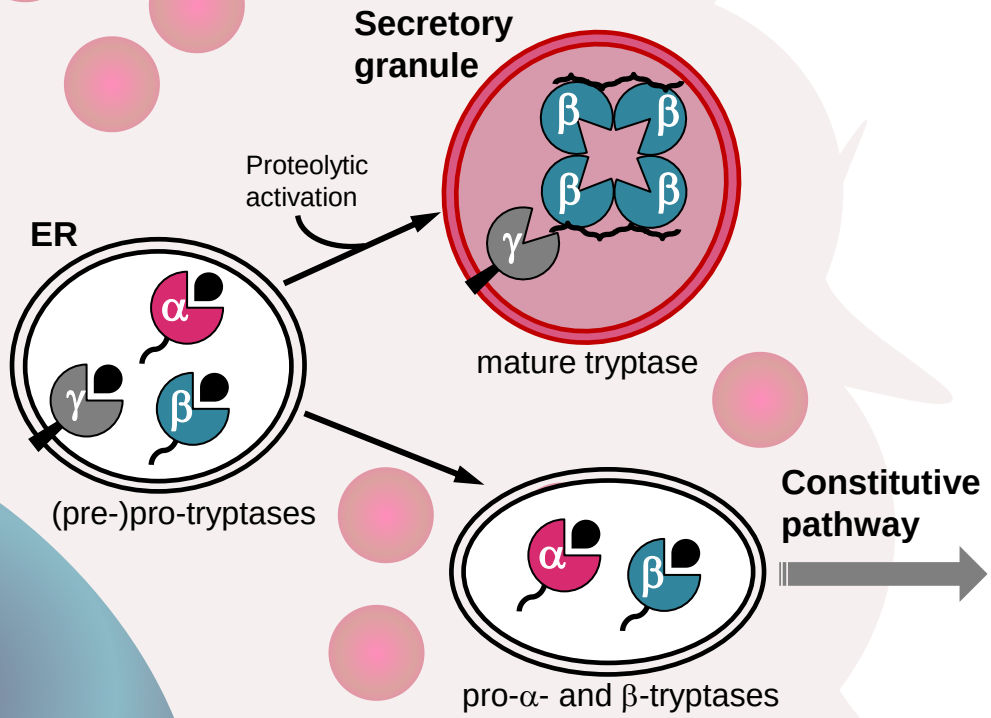
Lyons & Schwartz. *Mastocytosis.* 2019

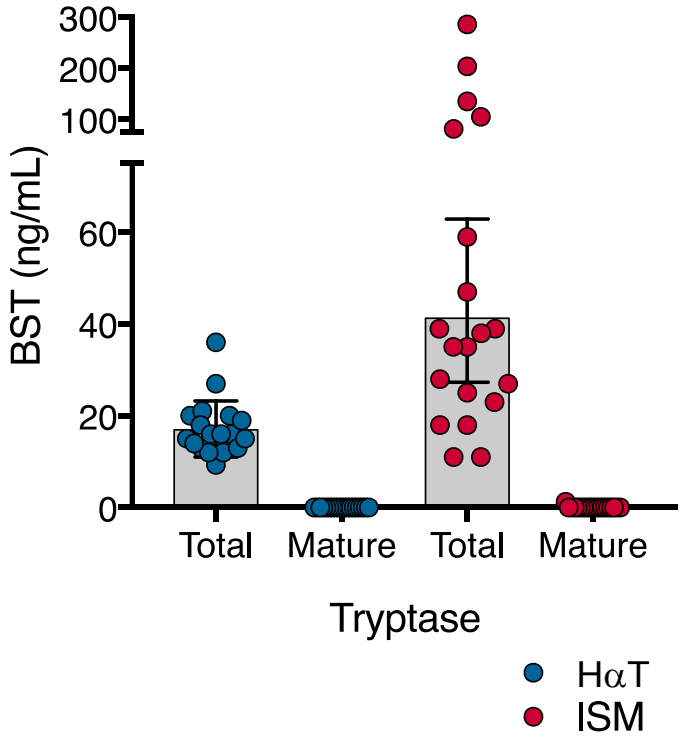
Tryptase: a biomarker for anaphylaxis and myeloid dyscrasias



- Mast cell product released during IgE-mediated reactions
- Myeloid diseases
  - Clonal proliferative disease
    - Mastocytosis
  - Myeloid dysplasia/neoplasia
- Genetic disorders affecting the mast cell compartment
  - Hereditary alpha-tryptasemia
  - *GATA2* haploinsufficiency
  - PLAID-associated *PLCG2* mutations

Basal serum tryptase (BST) is comprised of enzymatically inactive pro-tryptases

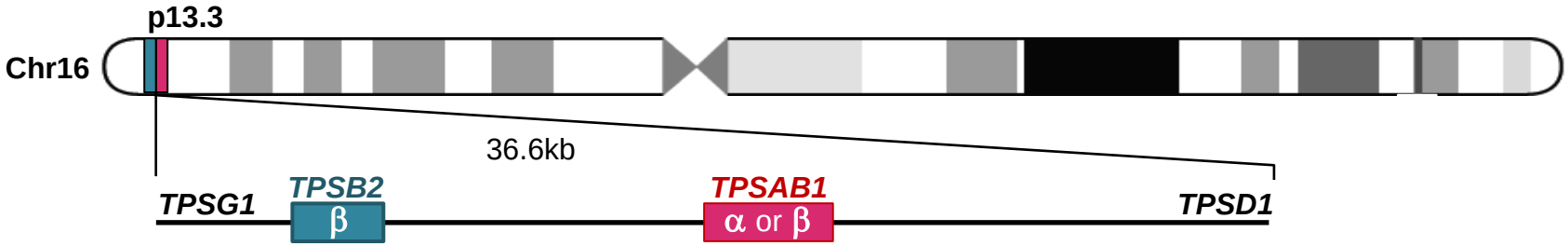




Mateja et al., Lyons. *JACI.* 2021

vons. *JAC.* 2018

Hereditary α-tryptasemia: genetic trait caused by *TPSAB1* replications

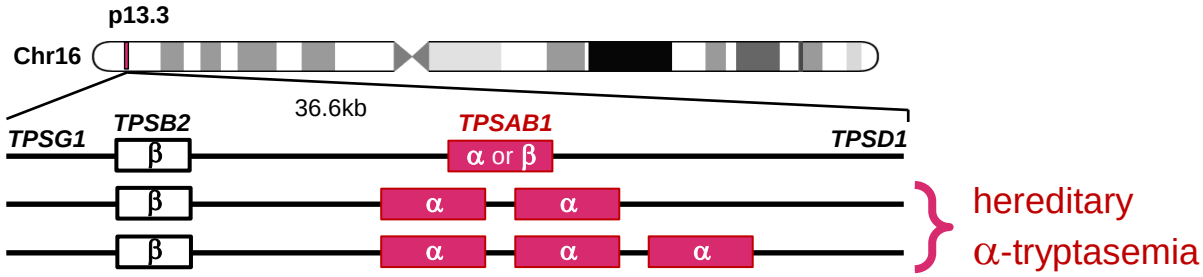


Canonical tryptase genotypes

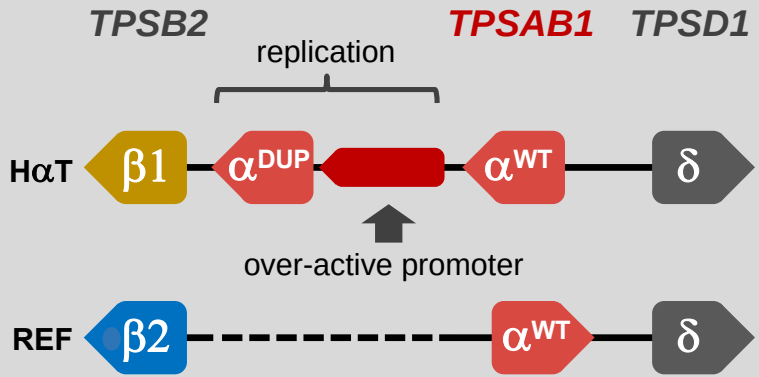
β/β, β/β = 4β:0α	30%
α/β, β/β = 3β:1α	44%
α/β, α/β = 2β:2α	21%

Lyons et al. *Nat Genet.* 2016

Lyons et al. *Nat Genet.* 2016

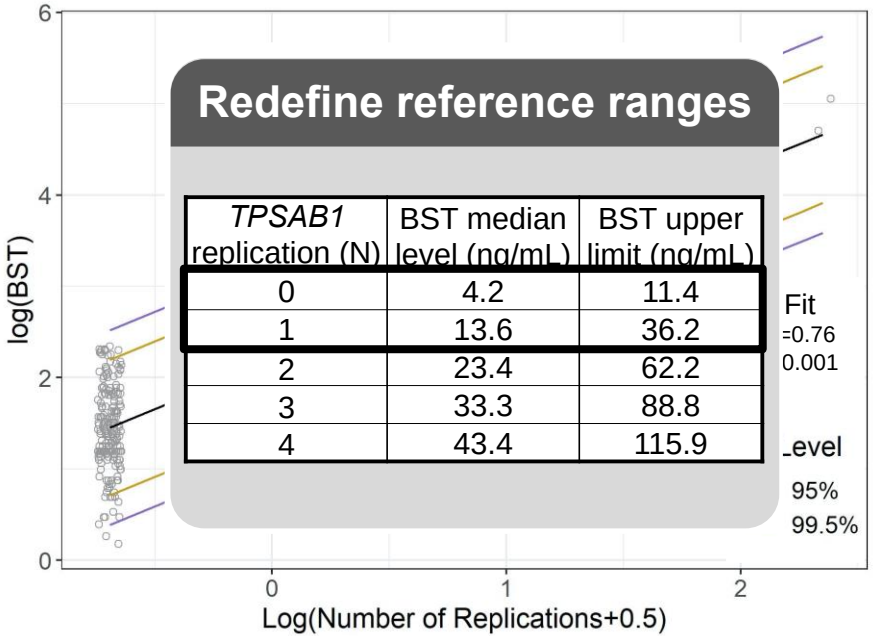


Long-read sequencing and assembly



Redefine reference ranges

TPSAB1 replication (N)	BST median level (ng/mL)	BST upper limit (ng/mL)
0	4.2	11.4
1	13.6	36.2
2	23.4	62.2
3	33.3	88.8
4	43.4	115.9

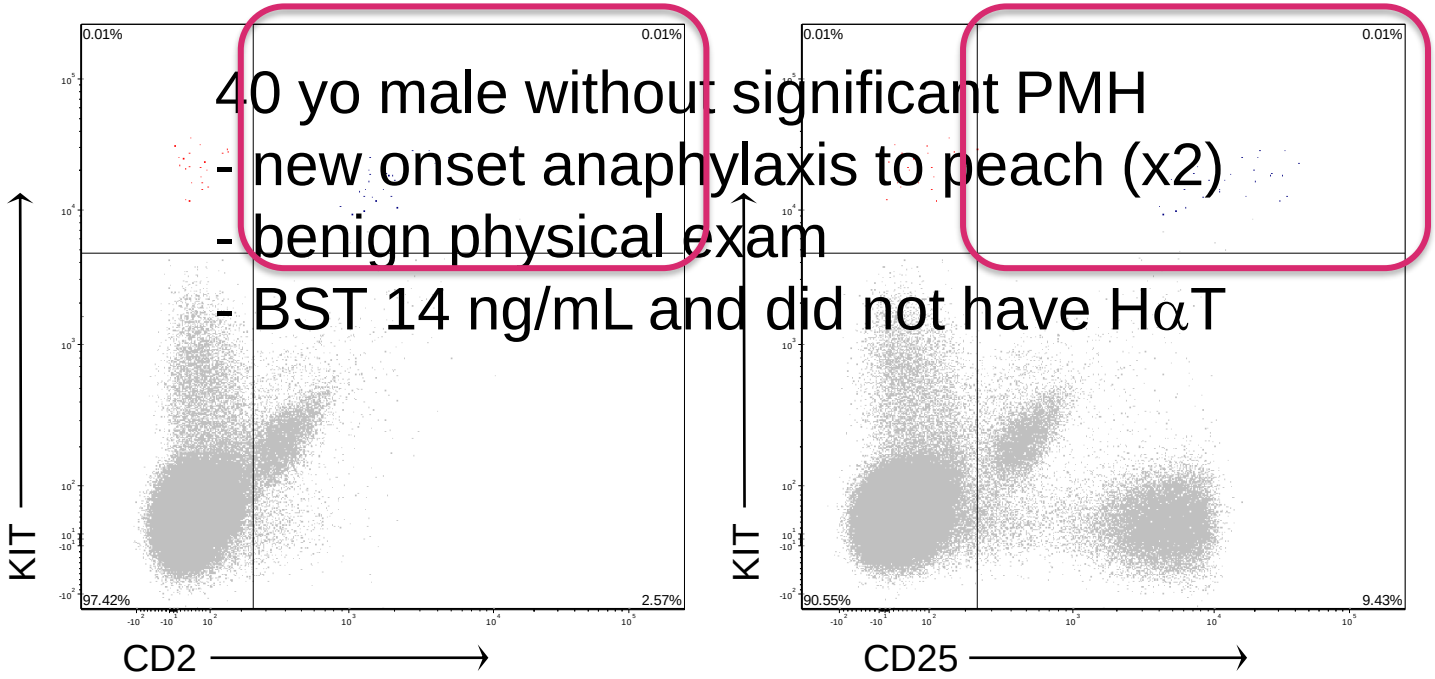


Chovanec et al., Lyons. *Blood Adv*. 2022

Lyons et al. *Nat Genet.* 2016

Elevated BST in the absence of HaT identifies clonal mast cell disease

40 yo male without significant PMH
- new onset anaphylaxis to peach (x2)
- benign physical exam
- BST 14 ng/mL and did not have HαT

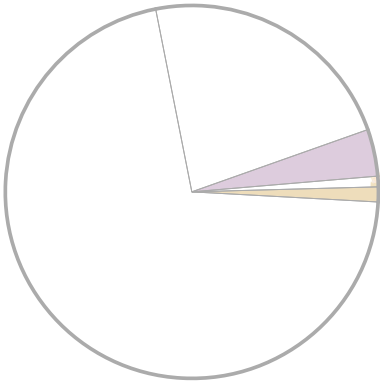


KIT p.D816V positive

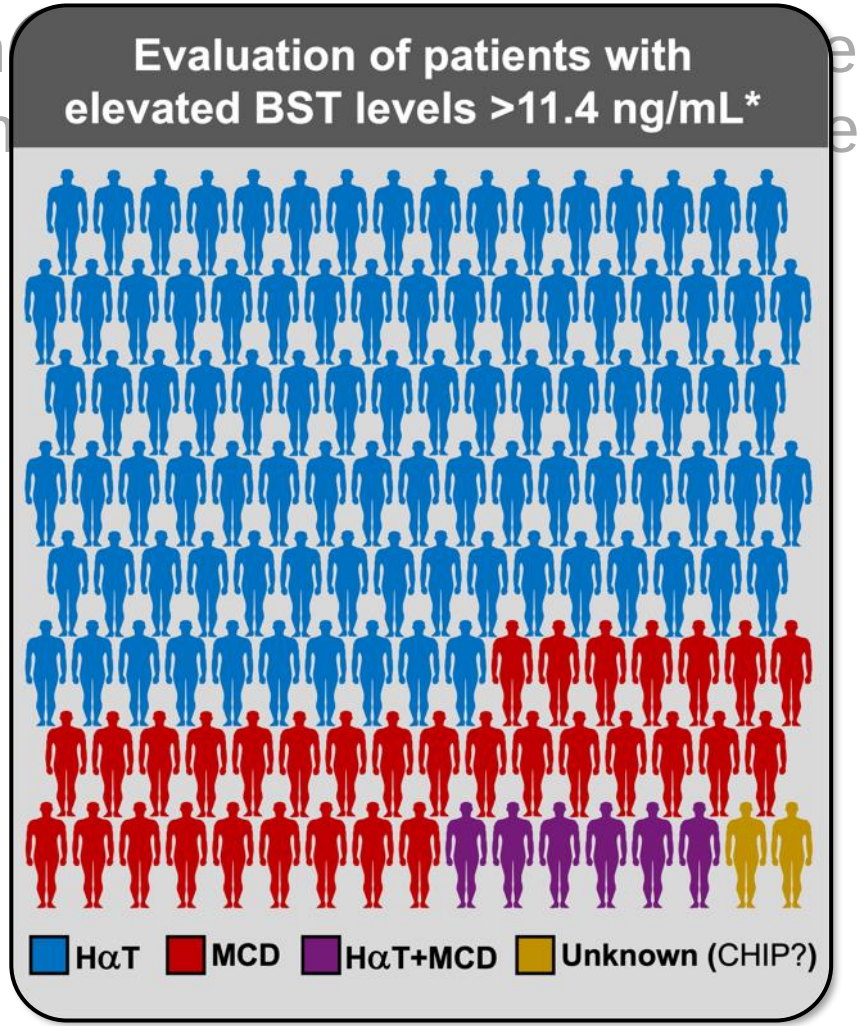
Diagnosis = MMAS

Elevated BST in es indolent or evolvin esis

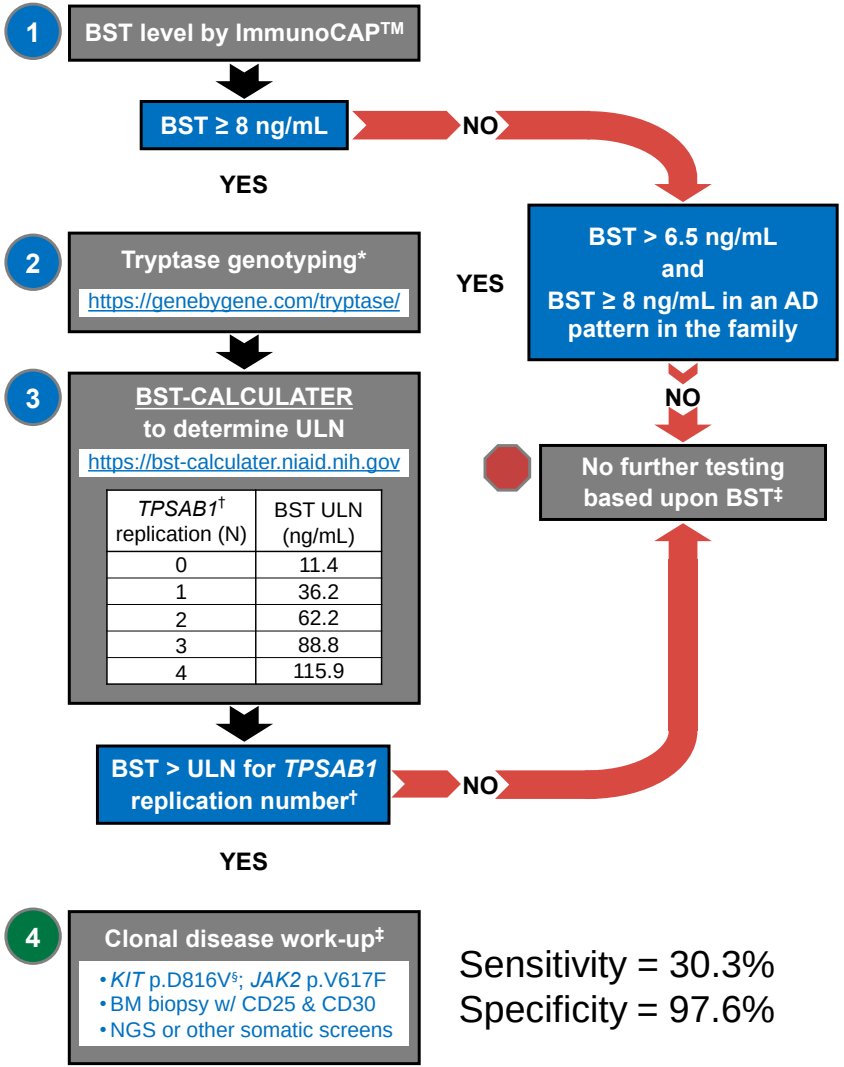
BST ≥ 8ng/mL
(N=671)



HαT
HαT + SM
SM
Idiosyncratic



Chovanec et al., Lyons. *Blood Adv*. 2022



Sensitivity = 30.3%
Specificity = 97.6%

Chovanec et al., Lyons. *Blood Adv.* 2022
McMurray et al. *Blood.* 2024

HαT impacts the specificity of using BST as a minor clinical criterion for diagnosing systemic mastocytosis

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[b]Invalid when another clonal myeloid disorder is present

Lyons & Schwartz. *Mastocytosis.* 2019

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BST CALCULATER **B**asal **S**erum **T**ryptase **C**linical cut-off **A**ssigned by **L**ocus **C**opy number of **U**TR-**L**inked element and **A**ssociated **T**PSAB1 **E**ncoded **R**eplication

https://bst-calculater.niaid.nih.gov

Chovanec et al., Lyons. *medRxiv.* 2022

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BST CALCULATER Basal Serum Tryptase Clinical cut-off Assigned by Locus Copy number of UTR-Linked element and Associated TPSAB1 Encoded Replication

Alpha copy number:

2

Beta copy number:

3

BST (ng/mL) (Optional):

Prediction Interval

99.5%

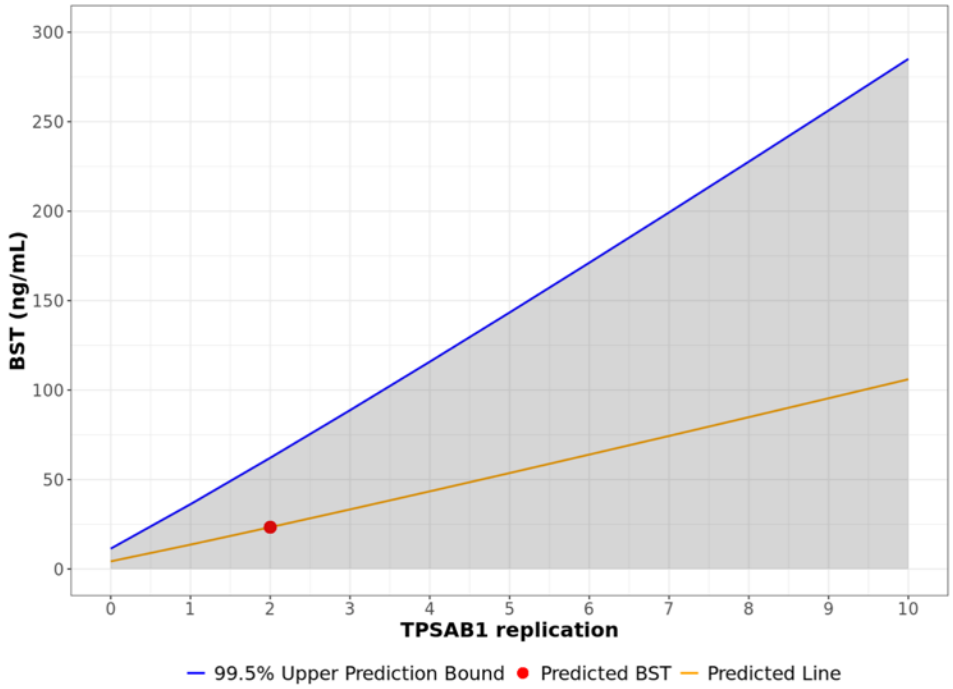
Work-up for Mastocytosis (Optional):

- Negative
- Positive
- Not Performed

Note: Significantly impaired renal function can also increase BST, and may impact this accuracy of this model.

Analyze my data | Reset

The predicted BST is 23.3642 ng/mL; The 99.5% upper prediction bound is 62.1517 ng/mL



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BST CALCULATER

Basal **S**erum **T**ryptase **C**linical cut-off **A**ssigned by **L**ocus **C**opy number of **U**TR-Linked element and **A**ssociated **T**PSAB1 **E**ncoded **R**eplication

Alpha copy number:

3

Beta copy number:

3

BST (ng/mL) (Optional):

35

Prediction Interval

99.5%

Work-up for Mastocytosis (Optional):

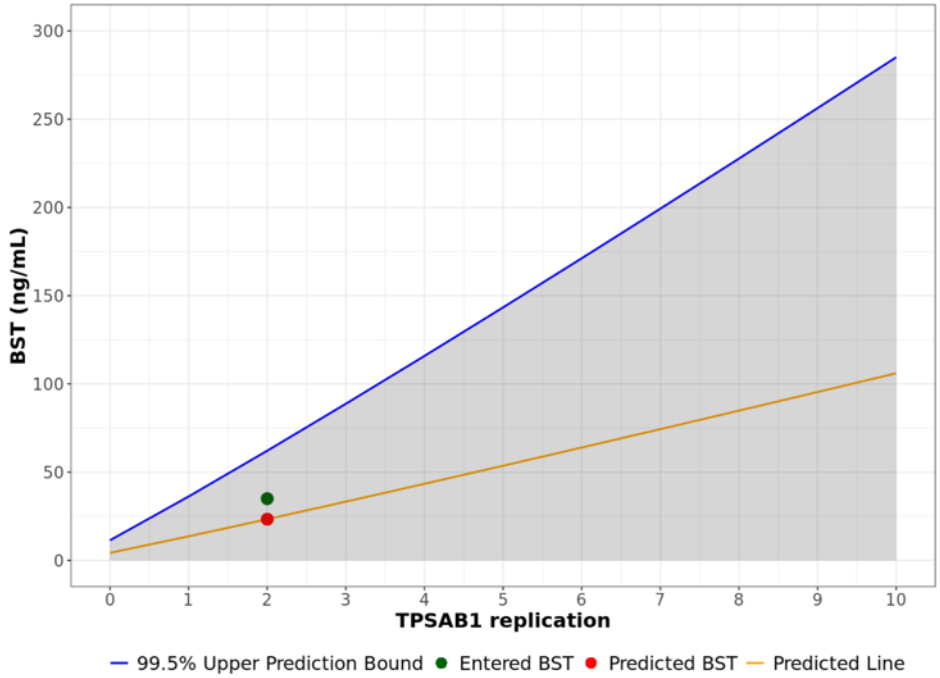
- Negative
- Positive
- Not Performed

Note: Significantly impaired renal function can also increase BST, and may impact this accuracy of this model.

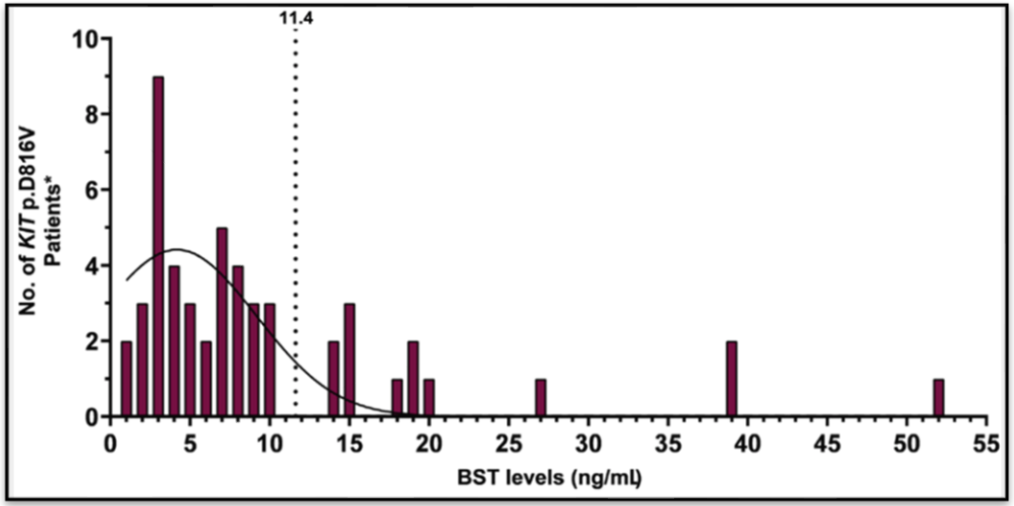
Analyze my data | Reset

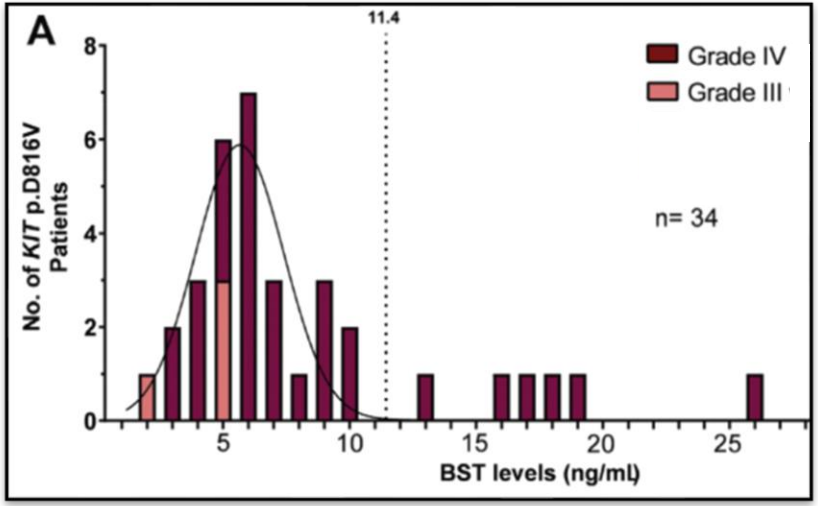
The predicted BST is 23.3642 ng/mL; The 99.5% upper prediction bound is 62.1517 ng/mL

The BST is within the prediction interval



Individuals with severe Hymenoptera reactions and *KIT* p.D816V frequently have normal BST





Lyons et al. *JACI*. 2020
Šelb et al. *JACI.* 2021

Scoring systems to risk stratify patients for BM biopsy

Table 8. Scoring systems used to stratify individuals with suspected clonal mast cell disease

Parameter	REMA [124, 199]	NICAS [65]
Gender	male +1	male +1
	female -1	female -1
Clinical Symptoms	pre-/syncope +3	syncope +3
	angioedema absent +1	angioedema absent +1
	urticaria absent +1	-
	pruritus absent +1	-
	flushing -1	flushing -1
	angioedema -1	-
	urticaria -1	urticaria +1
	pruritus -1	-
BST level (ng/mL)	>25 ng/mL +2	>11.4 ng/mL +1
	<15 ng/mL -1	<11.4 ng/mL -1
***KIT* p.D816V**	-	detected +3
	-	undetected -1

REMA - Red Española de Mastocitosis (Spanish Mastocytosis Network); NICAS - NIH Idiopathic Clonal Anaphylaxis Score; BST – basal serum tryptase. Total score ≥2 is associated with clonal disease in both scoring systems.

Park & Lyons. *Allergic and Immunologic Diseases. 2022*

Additional biomarkers

Table 6. Mast cell mediator tests to support the diagnosis of clonal MCAD or anaphylaxis

Analyte	Preferred method	Reference range	Level consistent with*: anaphylaxis	Level consistent with*: clonal MCAD	CLIA Laboratories
Total tryptase	serum/plasma†	≤11.4 ng/mL	≥20% + 2ng/mL over BST	>20 ng/mL‡	multiple
Mature tryptase	serum/plasma†	<1 ng/mL	≥1 ng/mL	NA	one‖
N-methylhistamine§	24-hour urine	0-5yo: 120-510 mcg/g Crt 6-16yo: 70-330 mcg/g Crt >16yo: 30-200 mcg/g Crt	≥2-fold over baseline¶	>200 ng/mg Crt¶	multiple
2,3-dinor-11β $PGF_{2\alpha}$§	24-hour urine	<5,205 pg/mg Crt	≥4-fold over baseline¶	>3,263 pg/mg Crt#	one Δ
LTE_4	24-hour urine	<104 pg/mg Crt	≥10-fold over baseline¶	>104 pg/mg Crt¶,#	one Δ

Park & Lyons. *Allergic and Immunologic Diseases. 2022*

Conclusions

- HαT is a common genetic trait caused by increased α-tryptase encoding *TPSAB1* copy number
- Elevated BST when encountered clinically is most often due to HαT
- Increased BST in HαT results from increased production of ‘normal’ alpha-tryptase
- *TPSAB1* replication number (when encoding α-tryptase) defines clinical reference ranges for BST
- BST with genotyping and *KIT* p.D816V are the two most useful biomarkers to screen for clonal mast cell disease
- BST >8ng/mL is uncommon
- BST >11.4ng/mL when HαT is not present likely represents a clonal myeloid disorder
- BST levels are frequently normal in patients with clonal mast cell disease and severe HVA; *KIT* p.D816V should be routinely sent