

Bone marrow tryptase as a possible diagnostic criterion for adult systemic mastocytosis

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Summary

Background Mastocytosis is difficult to diagnose, especially when systemic mast cell activation symptoms are not present or involve only one extracutaneous organ.

Objective The main objective was to evaluate the accuracy of the bone marrow tryptase level in the diagnosis of systemic mastocytosis in patients with a clinical suspicion of mastocytosis.

Methods We included all adult patients evaluated in our centre between December 2009 and 2013 for suspected mastocytosis as part of a standardized procedure and who had a bone marrow and serum tryptase assay on the same day. The diagnosis of systemic mastocytosis was established on the basis of the World Health Organization criteria as the gold standard. The accuracy of the bone marrow tryptase level in the diagnosis of systemic mastocytosis was assessed by a receiver operating characteristics curve analysis. The different sensitivity and specificity values, corresponding to the set of possible bone marrow tryptase level cut-off values, were estimated with 95% confidence intervals.

Results Seventy-three patients were included. The diagnosis of systemic mastocytosis was established in 43 patients (58.9%). The median bone marrow tryptase level was 423 µg/L [95% CI: 217–868] in the systemic mastocytosis group and 7.5 µg/L [95% CI: 4.6–17.1] in the non-systemic mastocytosis group ($P < 0.001$). A cut-off value of 50 µg/L for bone marrow tryptase identified systemic mastocytosis with a sensitivity of 93.0% [95% CI: 80.9–98.5%] and a specificity of 90.0% [95% CI: 73.5–97.9%].

Conclusion and Clinical Relevance The bone marrow tryptase level appears to be a valuable diagnostic criterion for confirming systemic mastocytosis. If this diagnosis can reliably be excluded by evaluation of the bone marrow tryptase level, there would be no need to perform a bone marrow biopsy.

Keywords tryptase, bone marrow tryptase, mastocytosis, systemic mastocytosis, diagnostic criterion, diagnostic

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Introduction

Mastocytosis is characterized by clonal accumulation of mast cells in various organs [1]. Mastocytosis qualifies as a rare disease with an estimated worldwide prevalence

of 1/20 000–1/40 000 [2]. Mast cell activation syndrome (MCAS) is a recently described functional mast cell disorder, suggesting that the incidence of mast cell diseases may be higher [3–5]. In clinical practice, the diagnosis of systemic mastocytosis (SM) in

adults should be investigated in two situations: (1) presence of cutaneous involvement (CuI), and/or (2) presence of chronic or recurrent mast cell activation symptoms [6].

Mastocytosis is difficult to diagnose, especially when skin lesions are discrete or absent. Using the diagnostic criteria defined by the World Health Organization (WHO), mastocytosis can be divided into cutaneous mastocytosis (CM) and SM [1]. Systemic mastocytosis may be associated with complications such as osteoporotic fractures, cytopenia or mast cells activation symptoms [7]. When CuI is diagnosed clinically and histologically or by molecular biology, it is often difficult to determine whether extracutaneous involvement is present because biopsies of presumptively involved organs are difficult to perform or are frequently inconclusive [8, 9]. It is, however, essential to establish the mast cell infiltration in different tissues to better classify mast cell disease in each patient. New drugs including tyrosine kinase inhibitors may soon be available and could modify the management of SM [10]. Even indolent forms of SM can be disturbing for patients despite limited tissue mast cell infiltration due to mast cell activation symptoms [10–12]. At rest, mast cells secrete mainly α - and pro- β tryptase, while mature β -tryptase is released only in the case of degranulation. Usually, the combined tryptase species, or total tryptase, are measured by standard laboratory methods. Serum tryptase level (ST) above 20 $\mu\text{g/L}$ is a minor criterion for SM [1].

Proelss et al. [13] have suggested that measurement of tryptase in bone marrow aspirate (BMA) could be useful for the diagnosis of SM. Measurement of tryptase produced by mast cells in BMA evaluates the presence of an excessive mast cell load outside the biopsied area, as the substance concerned is present initially at a high concentration in the marrow before being distributed to the general circulation. One potential limitation of this technique is that the absolute value of bone marrow tryptase level (MT) may not entirely reflect bone marrow mast cell load. Indeed, in practice, BMA is often contaminated with blood to varying degrees. By contrast, the calculation of the bone marrow tryptase/serum tryptase (MT/ST) ratio may reduce the issue of blood contamination during sampling.

The primary objective of this study was to evaluate the sensitivity and specificity of MT for the diagnosis of SM, using WHO diagnostic criteria as the reference standard. The secondary objectives were to evaluate the diagnostic accuracy of:

- (1) MT for the diagnosis of SM in two different populations: patients with CuI and patients without CuI.
- (2) MT/ST ratio for the diagnosis of SM in three different populations: patients with mast cell diseases, patients with CuI and patients without CuI.

Materials and methods

Patient population

We included all consecutive adult patients evaluated for a suspected diagnosis of mastocytosis between December 2009 and December 2013. Patients were referred due to CuI and/or presence of mast cell activation symptoms. As part of a standardized procedure, patients had MT measurement from BMA and ST from venous blood evaluated on the same day.

This study was approved by the Institutional Review Board of Necker Enfants-Malades Hospital and was carried out in accordance with the Helsinki Declaration. Written informed consent was obtained from all patients.

Diagnostic criteria

The diagnosis of SM was made using the diagnostic criteria defined by the WHO [1]. The major criterion was the presence of at least two dense aggregates of at least 15 mast cells identified by immunohistochemical study with anti-CD117 antibodies in bone marrow biopsy (BMB) sections. The minor criteria were as follows: (1) the presence of more than 25% of mast cells with atypical morphology; criterion searched on the histological sections of BMB; and/or on BM smear stained with toluidine blue, (2) abnormal expression of CD2 and/or CD25 by mast cells; criterion identified by immunohistochemistry study with anti-CD25 and anti-CD2 antibodies on BM smear and/or by multiparametric flow cytometry of mast cells, according to the method described by Valent et al. [3], (3) detection of a *KIT* point mutation at codon 816 (*D816V* mutation) by sequencing the 816 region of the *KIT* gene, determined by allele-specific quantitative polymerase chain reaction on ribonucleic acid/complementary deoxyribonucleic acid and (4) total ST $\geq 20 \mu\text{g/L}$, determined by a standardized fluoroenzyme immunoassay on a Phadia 250 automated analyzer (ThermoFisher Scientific; Villebon sur Yvette, France) [1]. SM was diagnosed if at least one major criterion +1 minor criterion or 3 minor WHO criteria were present [1, 14]. All diagnostic criteria were verified by C.B.L., a board-certified dermatologist specializing in mast cell diseases.

The diagnosis of CM was made when CuI was present without systemic involvement, as assessed after a complete clinical and laboratory evaluation [11]. Cutaneous mastocytosis was defined as recommended by Valent et al. on the basis of a typical skin eruption (major criterion) and one of the following two minor criteria: (1) monomorphic mast cell infiltrate consisting either of large aggregates of tryptase-positive mast cells (≥ 15 cells/cluster) or scattered mast cells exceeding 20 cells per microscopic high-power field ($\times 40$); (2)

detection of a *KIT* mutation at codon 816 in deoxyribonucleic acid extracted from a lesional skin biopsy [3].

The diagnosis of MCAS was made when all criteria proposed by the international consensus in 2012 were present [11]: (1) presence of typical symptoms of severe recurrent (or chronic) systemic mast cell activation symptoms, (2) involvement of mast cells documented by an increase in mast cell mediators in the blood during a crisis according to the formula: basic level of mast cell mediator + 20% + 2 µg/L, (3) symptoms responding to therapy with mast cell-stabilizing agents or drugs directed against mast cell mediator production, mediator release or mediator effects, (4) absence of a primary underlying mast cell disease (SM or CM), absence of other diagnoses that could explain the symptoms and/or IgE-mediated disease.

Tryptase analysis in serum and bone marrow aspirate

On the same day, venous blood and BMA were sampled into sodium heparin for ST and into ethylenediaminetetraacetic acid for ST and MT measurements, respectively. Bone marrow aspirate for MT measurement was obtained from iliac crest aspiration in the majority of patients. It was obtained from sternal puncture in patients who refused BMB. Total ST and total MT were determined by a standardized fluoroenzyme immunoassay on a Phadia 250 automated analyzer (ThermoFisher Scientific; Villebon sur Yvette) in accordance with the recommended procedures of the reagent supplier and Good Laboratory Practice (ISO standard 15189). Serum tryptase and marrow tryptase results were determined by P.A.P., who was blinded to the patient's history, including whether the patient had been evaluated for CuI or for mast cell activation symptoms.

Data collection and analysis

The following data were collected by M.E and C.B.L. in a specific case report form: demographic, clinical (SM, CM, MCAS) and laboratory (ST, MT and MT/ST ratio) patient characteristics.

Stata® SE Version 11.2 software (StataCorp, College Station, TX, United States) was used for statistical analysis by B.L., a Ph.D. methodologist, who specialized in diagnostic test accuracy. The distributions of ST values, MT values and MT/ST ratio were calculated in each group and compared using Mann–Whitney *U*-test. Correlation between MT value and presence of *KIT* mutation in bone marrow was investigated by the Wilcoxon Mann–Whitney *U*-test.

Evaluation of serum tryptase test accuracy

To evaluate ST diagnostic test accuracy, a cut-off value of 20 µg/L was chosen in the receiver operating

characteristics curve to distinguish between SM and non-SM patients, and between SM patients with CuI and CM patients [1]. To take into account the latest recommendations of the European Competence Network on Mastocytosis, a sensitivity analysis was performed using 15 µg/L as a cut-off value to distinguish between SM without CuI and MCAS [6].

Evaluation of bone marrow tryptase and bone marrow/serum tryptase ratio in the diagnosis of SM

Different cut-off values for MT and for MT/ST ratio were considered on the basis of the resulting receiver operating characteristics curve [15]. Receiver operating characteristics curves plotting sensitivity against 1-specificity were generated and the areas under the curve were calculated with 95% confidence intervals (95% CI). The areas under the curve obtained from MT and from MT/ST ratio were compared with the areas under the curve obtained from ST values in the sets of patients corresponding to three clinical questions: diagnosis of SM vs. non-SM, diagnosis of SM with CuI vs. CM, diagnosis of SM without CuI vs. MCAS. Examples of sensitivity and specificity values with 95% CI are given for cut-off values of ≥ 50 µg/L for the MT and ≥ 3.8 for the MT/ST ratio.

Evaluation of the predictive value of the MT and the MT/ST ratio

The positive predictive values and negative predictive values were calculated with the following formula: positive predictive values = sensitivity $\times$ prevalence/[sensitivity $\times$ prevalence + (1 – specificity) $\times$ (1 – prevalence)] and negative predictive values = specificity $\times$ (1 – prevalence)/[(1 – sensitivity) $\times$ prevalence] + specificity $\times$ (1 – prevalence) [16]. The prevalence of SM in patients with CuI ranges from 41% to 97% in the literature [17–21]. As the prevalence of SM in patients with CuI in our cohort was within this range, the SM prevalence in our cohort was used to estimate positive and negative predictive values.

Results

Patient population

Seventy-three patients were included. These were 33 male (45%) and 40 female patients (55%) with a mean age of 45 years (range 18–76 years). The diagnosis of SM was established in 43 patients (58.9%) by the WHO criteria: 38 patients (52.05%) with CuI and five patients (6.84%) without CuI. Among patients with SM, a patient had a low-grade B-cell lymphoma in the bone marrow. Of the 30 patients (41.1%) without SM, 14 (19.17%) had

CM and 16 (21.91%) had MCAS. Among patients with MCAS, one patient had a monoclonal MCAS (*D816V* mutation of *KIT*) and two patients a secondary MCAS: association with myelodysplasia in one patient and association with skin psoriasis in one patient.

Bone marrow biopsy was performed in 62 patients (85%) and yielded a diagnosis of SM in 19 patients. Thirteen patients with SM had a normal BMB indicating a sensitivity of 59% for BMB. In 24 patients, SM diagnosis was made in presence of three minor WHO criteria.

The presence of > 25% of mast cells with atypical morphology was shown in 24 of 43 patients with SM. Abnormal expression of CD25 and CD2 by mast cells was present in 10 of 14 and 8 of 10 patients with SM investigated, respectively, by immunohistochemistry analysis of BM smear and multiparametric flow cytometry. The detection of a *KIT* point mutation at codon 816 was positive in 38 of 40 patients with SM. No patient with CM or MCAS had a positive BMB or atypical morphology of mast cell on BM smear. Abnormal expression of CD25 and CD2 by mast cells was present in 0 of 4 and 4 of 8 patients with CM or MCAS investigated, respectively, by immunohistochemistry analysis of BM smear and multiparametric flow cytometry. Isolated detection of a *KIT* point mutation at codon 816 in the bone marrow was observed in three patients, 2 CM and one MCAS.

Serum tryptase

The median ST was 27 µg/L [95% CI: 22.9–36.0] in the SM group and 5.1 µg/L [95% CI: 3.9–7.1] in the non-SM group ($P < 0.001$; Fig. 1a). In the SM patients with CuI, the median ST level was 26.7 µg/L [95% CI: 22.9–35.0]. In CM patients without systemic involvement, the median ST was 5.1 µg/L [95% CI: 3.6–15.9] ($P < 0.001$; Fig. 1b). The median ST was 41 µg/L [95% CI: 13.6–160] in SM without CuI and 5.1 µg/L [95% CI: 3.9–7.1] in MCAS ($P = 0.005$; Fig. 1c).

A ST cut-off value of 20 µg/L differentiated SM from non-SM patients with a sensitivity of 79.1% [95% CI: 64–90%] and a specificity of 93.3% [95% CI: 77.9–99.2%], with an area under the curve of 0.918 [95% CI: 0.847–0.988] (Fig. 2a). For distinguishing SM patients with CuI from CM patients, the cut-off value of 20 µg/L for ST displayed a sensitivity of 78.9% [95% CI: 62.7–90.4%] and a specificity of 92.9% [95% CI: 66.1–99.8%], with an area under the curve of 0.942 [95% CI: 0.881–1.000] (Fig. 2b). A cut-off value of 15 µg/L distinguished between SM without CuI and MCAS with a sensitivity of 80.0% [95% CI: 28.4–99.5%] and a specificity of 81.3% [95% CI: 54.4–96.0%], with an area under the curve of 0.925 [95% CI: 0.808–1.000] (Fig. 2c).

Bone marrow tryptase

The median MT was 423 µg/L [95% CI: 217–868] in the SM patients and 7.5 µg/L [95% CI: 4.6–17.1] in the non-SM patients ($P < 0.001$; Fig. 1d). In the SM patients with CuI, the median MT was 441 µg/L [95% CI: 216–912], and in the CM patients, the median MT was 7.5 µg/L [95% CI: 4.3–27] ($P < 0.001$; Fig. 1e). The median MT level was 423 µg/L [95% CI: 30.8–1419] in SM patients without CuI and 7.2 µg/L [95% CI: 3.2–19.5] in MCAS ($P = 0.002$; Fig. 1f).

A cut-off value of 50 µg/L for MT was found to differentiate SM from non-SM with a sensitivity of 93.0% [95% CI: 80.9–98.5%] and a specificity of 90.0% [95% CI: 73.5–97.9%], with an area under the curve of 0.981 [95% CI: 0.958–1.000] (Fig. 2a). To distinguish between SM patients with CuI and CM patients, applying a cut-off value of 50 µg/L for MT gave a sensitivity of 94.7% [95% CI: 82.3–99.4%] and a specificity of 92.9% [95% CI: 66.1–99.8%], with an area under the curve of 0.977 [95% CI: 0.940–1.000] (Fig. 2b). Applying a cut-off value of 50 µg/L for the MT, to differentiate SM patients without CuI from MCAS patients gave a sensitivity of 80.0% [95% CI: 28.4–99.5%] and a specificity of 87.5% [95% CI: 61.7–98.4%], with an area under curve of 0.975 [95% CI: 0.916–1.000] (Fig. 2c).

In addition, all patients with ST level < 20 µg/L ($n = 9$) had BM tryptase > 50 µg/L.

Furthermore, the mean of MT level was, respectively, of 1974.21 µg/L (SD: 6514.2) and 74.9 µg/L (SD: 319.5) in patients with and without *KIT* mutation ($P = 0.0001$).

Bone marrow/serum tryptase ratio

The median MT/ST ratio was 15.53 [95% CI: 10.19–20.72] in the SM patients and 1.27 [95% CI: 1.12–1.38] in the non-SM patients ($P < 0.001$; Fig. 1g). In the SM patients with CuI, the median MT/ST ratio was 16.31 [95% CI: 10.54–23.64], and in CM patients, the median MT/ST ratio was 1.30 [95% CI: 1.07–2.23] ($P < 0.001$; Fig. 1h). The median MT/ST was 8.87 [95% CI: 2.26–20.72] in SM patients without CuI and 1.14 [95% CI: 1.08–1.47] in MCAS ($P = 0.004$; Fig. 1i).

A cut-off value of 3.8 for the MT/ST ratio was applied to differentiate SM from non-SM. It displayed a sensitivity of 95.3% [95% CI: 84.2–99.4%] and a specificity of 90.0% [95% CI: 73.5–97.9%], with an area under the curve of 0.954 [95% CI: 0.897–1.000] (Fig. 2a). To distinguish between SM patients with CuI and CM patients, the cut-off value of 3.8 gave a sensitivity of 97.4% [95% CI: 86.2–99.9%] and a specificity of 92.9% [95% CI: 66.1–99.8%], with an area under the curve of 0.940 [95% CI: 0.833–1.000] (Fig. 2b). A cut-off value of 3.8 to differentiate SM patients without CuI from MCAS patients gave a sensitivity of 80.0% [95% CI: 28.4–99.5%] and a

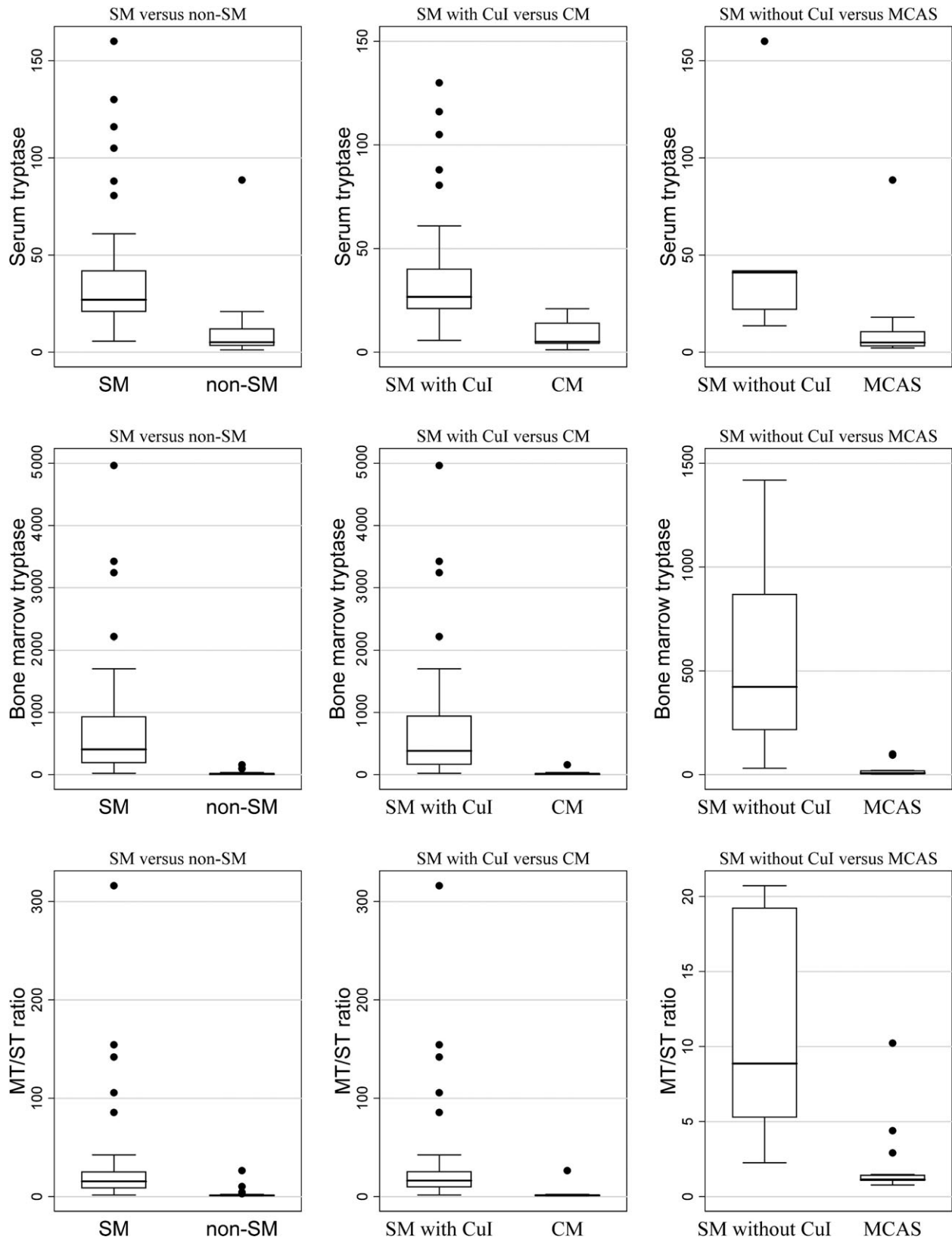


Fig. 1. Distribution of ST (panel a), MT (panel b) and MT/ST ratio (panel c) in the three groups of patients: SM vs. non-SM, SM with cutaneous involvement vs. CM and SM without cutaneous involvement vs. MCAS. ST, serum tryptase; MT, bone marrow tryptase level; MT/ST, bone marrow tryptase/serum tryptase; SM, systemic mastocytosis; CM, cutaneous mastocytosis; MCAS, mast cell activation syndrome.

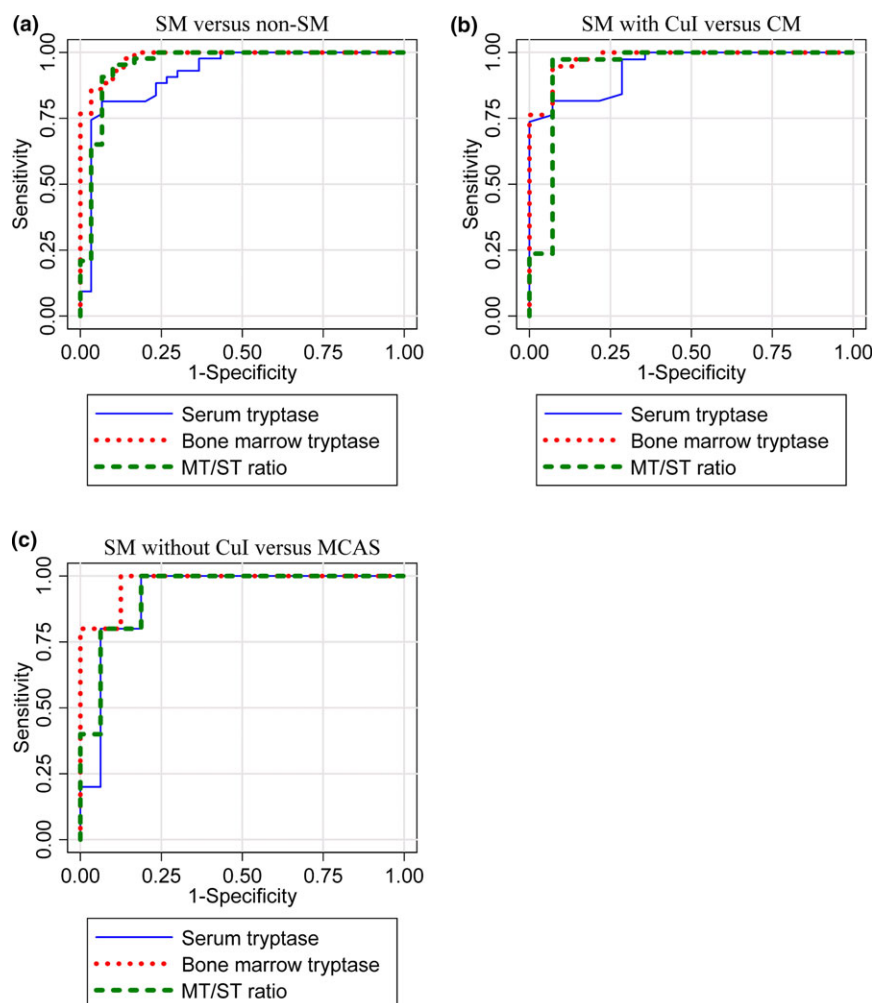


Fig. 2. ROC curves investigating the utility of the MT in identifying patients with SM. Panel a: SM among patients with suspected mastocytosis; panel b: SM among patients with cutaneous involvement; and panel c: SM among patients without cutaneous involvement. MT, bone marrow tryptase level; MT/ST ratio, bone marrow tryptase/serum tryptase ratio; CuI, cutaneous involvement; SM, systemic mastocytosis; ROC, receiver operating characteristic; AUROC, area under the ROC curve; 95% CI, 95% confidence interval.

specificity of 87.5% [95% CI: 61.7–98.4%], with an area under the curve of 0.938 [95% CI: 0.835–1.000] (Fig. 2c).

Table S1 summarizes the results of MT, ST and MT/ST ratio for all patients.

Areas under the curve comparisons

The areas under the curve obtained with MT were significantly higher than the areas under the curve obtained with ST levels when diagnosing SM vs. non-SM ($P = 0.05$; Fig. 2a). Analysis of MT/ST ratio provided no significant benefit over MT.

Positive predictive value and negative predictive value of the MT and the MT/ST ratio

The prevalence of SM in patients with a mast cell disease (SM, CM and MCAS) was 58.9%. The prevalence of

SM in patients with CuI was 73%. The prevalence of SM in patients with mast cell activation symptoms without CuI was 23.8%.

The positive and negative predictive values of a MT > 50 µg/L for a diagnosis of SM were, respectively, of 93.0% and 90.0%. For patients with CuI, the positive and negative predictive values of MT > 50 µg/L for a diagnosis of SM with CuI were, respectively, of 97.3% and 86.7%. For patients without CuI, the positive predictive and negative values of a MT > 50 µg/L for a diagnosis of SM without CuI were, respectively, of 66.7% and 93.3%.

The positive and negative predictive values of a MT/ST ratio > 3.8 for a diagnosis of SM were, respectively, of 93.2% and 93.1%. For patients with CuI, the positive and negative predictive values of a MT/ST ratio > 3.8 for a diagnosis of SM with CuI were, respectively, of 97.4% and 92.9%. For patients without CuI, the positive

predictive and negative values of a MT/ST ratio > 3.8 for a diagnosis of SM without CuI were, respectively, of 66.7% and 93.3%.

Table 1 summarizes the sensitivity, specificity, positive and negative predictive values, and area under the curve for ST, MT and MT/ST in the three groups of patients. All the results of the area under the curve for ST, MT and MT/ST in the patient's subgroups are presented in the supplemental data (Tables S3–S5).

Discussion

We report here the ability of MT and MT/ST ratio to detect systemic involvement in adults with a clinical suspicion of mastocytosis. A level of MT of 50 $\mu\text{g/L}$ or more or a MT/ST ratio of 3.8 or more is highly suggestive of SM, with a sensitivity over 93% for both and a similar specificity of 90.0%. To distinguish between SM and CM in patients with CuI, a MT of 50 $\mu\text{g/L}$ or more or a MT/ST ratio of 3.8 or more is still highly suggestive of SM with a sensitivity of, respectively, 94.7% and 97.4% and a specificity of 92.9% for both. A level of 50 $\mu\text{g/L}$ or more or a MT/ST ratio of 3.8 or more allows SM patients without CuI to be distinguished from MCAS patients with a sensitivity of 80.0% and a specificity of 87.5% for both tests.

In this study, we show also that the diagnostic properties of MT evaluate better than those of ST for

diagnosis of SM in both patients with and without CuI. However, in patients with MCAS, the diagnostic properties of both ST and MT are lower as compared to CM patients. MT/ST ratio appears to offer no benefits over MT for the diagnosis of SM. It is of note that in our cohort the diagnostic properties of ST for SM diagnosis are similar to those reported in the literature, showing a sensitivity of 78.9–80% and a specificity of 81–93% [22–24]. As tryptase is initially produced primarily in the bone marrow in SM, direct measurement of MT was expected to offer better ability to diagnosis SM as compared to ST. Moreover, as the genes encoding tryptase in humans are highly polymorphic, the production, maturation and secretion of tryptase may be reduced in some patients with particular genetic variants. This polymorphism of the tryptase gene (*TPS1/TPS2*) could explain the low ST in some patients with SM [25–27]. MT evaluation allows to diagnose SM in some patients with low ST: see the cases of patients with SM number #4a, #6, #7, #8, #9, #10, #12, #15 and #35 in Table S1.

The sensitivity of MT or MT/ST ratio to detect SM appears better than the one of BMB. Bone marrow biopsy showed a sensitivity of 68–80% for SM diagnosis in experienced centres [8, 9]. Due to the low frequency of mast cells in the marrow (< 0.002 – 0.05%) and the focal nature of the mast cell infiltrate [9], BMB cannot be considered a sensitive diagnostic criterion for SM despite being a major diagnostic criterion according

Table 1. Sensitivity, specificity, positive and negative predictive values, and AUROC for ST, MT and MT/ST ratio in three groups of patients: SM vs. non-SM, SM with CuI vs. CM and SM without CuI vs. MCAS

		ST		MT			MT/ST ratio		
		Value	95% CI	Value	95% CI	P-value*	Value	95% CI	P-value*
SM ($n = 43$) vs. non-SM ($n = 30$) Prevalence: 59%	Threshold	ST ≥ 20 $\mu\text{g/L}$		MT ≥ 50 $\mu\text{g/L}$			MT/ST ≥ 3.8		
	Se	79.1%	64.0–90.0%	93.0%	80.9–98.5%		95.3%	84.2–99.4%	
	Sp	93.3%	77.9–99.2%	90.0%	73.5–97.9%		90.0%	73.5–97.9%	
	PVP	94.4%		93.0%			93.2%		
	NVP	75.7%		90.0%			93.1%		
	AUROC	91.8%	84.7–98.9%	98.1%	95.8–100.0%	0.05	95.4%	89.7–100.0%	0.44
SM with CuI ($n = 38$) vs. MC ($n = 14$) Prevalence: 73%	Threshold	ST ≥ 20 $\mu\text{g/L}$		MT ≥ 50 $\mu\text{g/L}$			MT/ST ≥ 3.8		
	Se	78.9%	62.7–90.4%	94.7%	82.3–99.4%		97.4%	86.2–99.9%	
	Sp	92.9%	66.1–99.8%	92.9%	66.1–99.8%		92.9%	66.1–99.8%	
	PVP	96.8%		97.3%			97.4%		
	NVP	62.0%		86.7%			92.9%		
	AUROC	94.2%	88.1–100.0%	97.7%	94.1–100.0%	0.27	94.0%	83.3–100.0%	0.98
SM without CuI ($n = 5$) vs. MCAS ($n = 16$) Prevalence: 24%	Threshold	ST ≥ 15 $\mu\text{g/L}$		MT ≥ 50 $\mu\text{g/L}$			MT/ST ≥ 3.8		
	Se	80.0%	28.4–99.5%	80.0%	28.4–99.5%		80.0%	28.4–99.5%	
	Sp	81.3%	54.4–96.0%	87.5%	61.7–98.4%		87.5%	61.7–98.4%	
	PVP	57.1%		66.7%			66.7%		
	NVP	92.9%		93.3%			93.3%		
	AUROC	92.5%	80.8–100.0%	97.5%	91.6–100.0%	0.26	93.8%	83.5–100.0%	0.86

*AUROC comparison with AUROC obtained from ST level.

ST, serum tryptase level; MT, bone marrow tryptase level; MT/ST ratio, bone marrow tryptase/serum tryptase ratio; PPV, positive predictive value; NPV, negative predictive value; SM, systemic mastocytosis; CM, cutaneous mastocytosis; MCAS, mast cell activation syndrome; CuI, cutaneous involvement; Se, sensitivity; Sp, specificity; AUROC, area under the receiver operating characteristic curve; 95% CI, 95% confidence interval.

to WHO. In addition, BMB is a painful procedure with carries a risk of bleeding. According to the literature and our experience, the acceptability of BMA is better than the one of BMB [28, 29].

Other minor laboratory criteria of SM have been proposed. Their diagnostic properties have not been fully established [3, 11, 30–35]. Among them, only flow cytometry for measuring CD2, CD25 expression on mast cells in BMA displayed high sensitivity [11, 33].

Recently, it was suggested that detection of *KIT D816V* mutation in blood cells could be a diagnostic tool for SM. Future studies are needed to determine the diagnostic value of MT in BMA vs. *D816V* mutation in blood [36].

There are potential limitations of our work. First, MT may vary according to the site of aspiration, the volume of aspiration, the contamination with blood and the stromal composition of the marrow. The low variability of MT in non-SM patients suggests that the MT evaluation is highly reproducible. Secondly, the single-centre recruitment and the relatively small sample size indicate that the results need to be replicate in a prospective large multicentre study. One potential limitation of tryptase level in blood or bone marrow as an assessment of mast cell load is that SM patients with α - and β -tryptase gene polymorphisms may have normal tryptase level in blood and bone marrow [8]. However, all patients with SM and ST < 20 $\mu\text{g/L}$ had the BM

tryptase level > 50 $\mu\text{g/L}$. One other potential limitation of our study is that no comparison of TM levels in healthy subjects and in patients with other haematologic diseases was performed. However, the clinical context of interest is diagnosis of SM in patients presenting with CM and/or mast cell activation symptoms. MT evaluation has better diagnostic properties than ST. If these findings are confirmed, the diagnosis of SM could be facilitated, possibly leading to a revision of the WHO diagnostic criteria. As a result, the management strategy for a given patient would be improved.

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Conflict of interest

The authors declare no conflict of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Bone marrow tryptase level, serum tryptase level and bone marrow tryptase/serum tryptase ratio in different patient groups.

Table S2. The area under the curve for serum tryptase level, bone marrow tryptase level and MT/ST ratio in SM group vs. non-SM group.

Table S3. The area under the curve for serum tryptase level, bone marrow tryptase level and MT/ST ratio in SM with CuI group vs. non-CM group.

Table S4. The area under the curve for serum tryptase level, bone marrow tryptase level and MT/ST ratio in SM without CuI group vs. MCAS group.

Table S1: Bone marrow tryptase level, serum tryptase level and bone marrow tryptase/serum tryptase ratio in different patient groups.

Systemic mastocytosis					Cutaneous mastocytosis				
Patient	Age (years)	MT (µg/L)	ST (µg/L)	MT/ST ratio	Patient	Age (years)	MT (µg/L)	ST (µg/L)	MT/ST ratio
#1	29	405	61	6.64	*1	21	1.6	1,2	1.33
#2	53	12 245	116	105.56	*2	65	4.82	5.29	0.91
#3	46	477	23.6	20.21	*3	25	7.82	4.23	1.85
#4	33	99	10	9.90	*3	56	20,8	16	1.30
#5	29	190	20.4	9.31	*4	46	11.3	4.9	2.31
#6	30	21.7	5.7	3.81	*5	45	18	14	1.29
#7	34	107	6.26	17.09	*6	58	4.58	4.28	1.07
#8	47	331	14	23.64	*7	20	158	5.99	26.38
#9	40	77.4	18.1	4.28	*8	32	4.26	3.39	1.26
#10	41	29	6.2	4.68	*9	69	27	21	1.29
#11	53	217	41	5.29	*10	19	5.84	3.57	1.64
#12	73	30.8	13.6	2.26	*11	47	7.17	5.48	1.31
#13	69	3242	21	154.38	*12	52	35.4	15.9	2.23
#14	45	777	26.4	29.43	*13	35	4.2	4.67	0.90
#15	62	137	13	10.54					
#16	30	665	34	19.56					
#17	47	133	27	4.93	"1	73	17	3.87	4.39
#18	32	356	29	12.28	"2	64	3.43	3.24	1.06

#19	41	496	46	10.78	"3	56	2.94	2.62	1.12
#20	50	210	46.9	4.48	"4	61	6.56	6.1	1.08
#21	40	190	22.9	8.30	"5	49	2.53	3.24	0.78
#22	62	3423	40	85.58	"6	34	3.24	3.43	0.94
#23	51	1165	48	24.27	"7	44	4.32	2.93	1.47
#24	70	716	32.5	22.03	"8	46	19.5	18	1.08
#25	55	912	36	25.33	"9	36	4.89	3.88	1.26
#26	45	930	35	26.57	"10	54	14	12	1.17
#27	41	868	41.9	20.72	"11	52	20.7	7.1	2.92
#28	56	1419	160	8.87	"12	29	17.1	15.8	1.08
#29	33	274	25.1	10.92	"13	43	2.99	2.16	1.38
#30	60	1702	40.1	42.44	"14	57	7.84	6.7	1.17
#31	56	41085	130	316.04					
#32	53	4965	35	141.86					
#33	40	485	23.7	20.46					
#34	56	245	22	11.14	"15	75	94	9.19	10.23
#35	60	102	8.87	11.50	"16	71	99.4	88.63	1.12
#36	54	2215	88	25.17					
#37	76	423	22	19.23					
#38	50	953	24	39.71					
#39	53	216	21.2	10.19					

#40	24	52.8	31	1.70
#41	23	1486	80.6	18.44
#42	30	264	26	10.15
#43	38	1631	105	15.53

MT: bone marrow tryptase level; ST: serum tryptase level; MT/ST ratio: bone marrow tryptase/serum tryptase ratio; MCAS: mast cell activation syndrome.

Table S2: The area under the curve for serum tryptase level, bone marrow tryptase level and MT/ST ratio in SM group versus non-SM group.

ST			MT			MT/STratio		
Cutpoint	Sensitivity (%)	Specificity (%)	Cutpoint	Sensitivity (%)	Specificity (%)	Cutpoint	Sensitivity (%)	Specificity (%)
≥1.2	100.0	0.0	≥1.6	100.0	0.0	≥0.7	100.0	0.0
≥2.1	100.	3.3	≥2.5	100.0	3.3	≥0.8	100.0	3.3
≥2.6	100.0	6.6	≥2.94	100.0	6.6	≥0.91	100.0	6.6
≥2.9	100.0	10.0	≥2.99	100.0	10.0	≥0.94	100.0	10.0
≥3.2	100.0	13.3	≥3.2	100.0	13.3	≥1.05	100.0	13.3
≥3.3	100.0	20.0	≥3.4	100.0	16.6	≥1.07	100.0	16.6
≥3.4	100.0	23.3	≥4.2	100.0	20.0	≥1.07	100.0	20.0
≥3.5	100.0	26.6	≥4.26	100.0	23.3	≥1.08	100.0	23.3
≥3.87	100.0	30.0	≥4.3	100.0	26.6	≥1.08	100.0	26.6
≥3.88	100.0	33.3	≥4.5	100.0	30.0	≥1.12	100.0	30.0
≥4.23	100.0	36.6	≥4.82	100.0	33.3	≥1.12	100.0	33.3
≥4.28	100.0	40.0	≥4.89	100.0	36.6	≥1.16	100.0	36.6
≥4.6	100.0	43.3	≥5.8	100.0	40.0	≥1.17	100.0	40.0
≥4.9	100.0	46.6	≥6.5	100.0	43.3	≥1.25	100.0	43.3
≥5.2	100.0	50.0	≥7.1	100.0	46.6	≥1.26	100.0	46.6
≥5.4	100.0	53.3	≥7.82	100.0	50.0	≥1.28	100.0	50.0
≥5.7	100.0	56.6	≥7.84	100.0	53.3	≥1.3	100.0	56.6
≥5.9	97.6	56.6	≥11.3	100.0	56.6	≥1.30	100.0	60.0
≥6.1	97.6	60.0	≥14	100.0	60.0	≥1.33	100.0	63.3
≥6.2	97.6	63.3	≥17	100.0	63.3	≥1.38	100.0	66.6
≥6.2	95.3	63.3	≥17.1	100.0	66.6	≥1.4	100.0	70.0
≥6.7	93.0	63.3	≥18	100.0	70.0	≥1.6	100.0	73.3
≥7.1	93.0	66.6	≥19.5	100.0	73.3	≥1.7	100.0	76.6

≥ 8.8	93.0	70.0	≥ 20.7	100.0	76.6	≥ 1.8	97.6	76.6
≥ 10.3	90.7	70.0	≥ 20.8	100.0	80.0	≥ 2.2	97.6	80.0
≥ 10.6	90.7	73.3	≥ 21.7	100.0	83.3	≥ 2.26	97.6	83.3
≥ 12	88.3	73.3	≥ 27	97.6	83.3	≥ 2.3	95.3	83.3
≥ 13	88.3	76.6	≥ 29	97.6	86.6	≥ 2.9	95.3	86.6
≥ 13.6	86.0	76.6	≥ 30.8	95.3	86.6	≥ 3.8	95.3	90.0
≥ 14	83.7	76.6	≥ 35.4	93.0	86.6	≥ 4.2	93.0	90.0
≥ 15.8	81.4	80.0	≥ 52.8	93.0	90.0	≥ 4.3	90.7	90.0
≥ 15.9	81.4	83.3	≥ 77.4	90.7	90.0	≥ 4.4	90.7	93.3
≥ 16	81.4	86.6	≥ 94	88.3	90.0	≥ 4.6	88.3	93.3
≥ 18	81.4	90.0	≥ 99	88.3	93.3	≥ 4.9	86.0	93.3
≥ 18.1	81.4	93.3	≥ 99.4	86.0	93.3	≥ 5.2	83.7	93.3
≥ 20.4	79.0	93.3	≥ 102	86.0	96.6	≥ 6.6	81.4	93.3
≥ 21	76.7	93.3	≥ 107	83.7	96.6	≥ 6.9	79.0	93.3
≥ 21.2	74.4	96.6	≥ 133	81.4	96.6	≥ 7.8	76.7	93.3
≥ 22	72.0	96.6	≥ 137	79.0	96.6	≥ 8.2	76.7	96.6
≥ 22.9	67.4	96.6	≥ 158	76.7	96.6	≥ 9.3	74.4	96.6
≥ 23.7	65.1	96.6	≥ 190	76.7	100.0	≥ 10.15	72.0	96.6
≥ 24	62.7	96.6	≥ 210	72.0	100.0	≥ 10.18	69.7	96.6
≥ 25.1	60.4	96.6	≥ 216	69.7	100.0	≥ 10.5	67.4	96.6
≥ 25.2	58.1	96.6	≥ 217	67.4	100.0	≥ 10.7	65.1	96.6
≥ 26	55.8	96.6	≥ 245	65.1	100.0	≥ 10.9	62.7	96.6
≥ 26.4	53.4	96.6	≥ 264	62.7	100.0	≥ 10.9	60.4	96.6
≥ 27	51.1	96.6	≥ 274	60.4	100.0	≥ 11.1	58.1	96.6
≥ 29	48.8	96.6	≥ 331	58.1	100.0	≥ 11.4	55.8	96.6
≥ 31	46.5	96.6	≥ 356	55.8	100.0	≥ 12.1	53.4	96.6
≥ 32.5	44.1	96.6	≥ 405	53.4	100.0	≥ 12.2	51.1	96.6
≥ 34	41.8	96.6	≥ 423	51.1	100.0	≥ 15.5	48.8	96.6

≥35	39.5	96.6		≥477	48.8	100.0		≥17.0	46.5	96.6
≥36	34.8	96.6		≥485	46.5	100.0		≥18.4	44.1	96.6
≥40	32.5	96.6		≥496	44.1	100.0		≥19.2	41.8	96.6
≥40.1	30.2	96.6		≥665	41.8	100.0		≥19.5	39.5	96.6
≥41	27.9	96.6		≥716	39.5	100.0		≥20.4	37.2	96.6
≥41.9	25.5	96.6		≥777	37.2	100.0		≥20.7	34.8	96.6
≥46	23.2	96.6		≥868	34.8	100.0		≥22.0	32.5	96.6
≥46.9	20.9	96.6		≥912	32.5	100.0		≥23.6	30.2	96.6
≥48	18.6	96.6		≥930	30.2	100.0		≥24.2	27.9	96.6
≥61	16.2	96.6		≥953	27.9	100.0		≥25.1	25.5	96.6
≥80.6	13.9	96.6		≥1165	25.5	100.0		≥25.3	23.2	96.6
≥88	11.6	96.6		≥1419	23.2	100.0		≥26.3	20.9	96.6
≥88.6	9.3	96.6		≥1486	20.9	100.0		≥26.5	20.9	100.0
≥105	9.3	100.0		≥1631	18.6	100.0		≥29.4	18.6	100.0
≥116	6.9	100.0		≥1702	16.2	100.0		≥39.7	16.2	100.0
≥130	4.6	100.0		≥2215	13.9	100.0		≥42.4	13.9	100.0
≥145	2.3	100.0		≥3242	11.6	100.0		≥85.5	11.6	100.0
>145	0.0	100.0		≥3423	9.3	100.0		≥105.5	9.3	100.0
				≥4965	6.9	100.0		≥141.8	6.9	100.0
				≥12245	4.6	100.0		≥154.3	4.6	100.0
				≥41085	2.3	100.0		≥316.0	2.3	100.0
				>41085	0.0	100.0		>316.0	0.0	100.0

MT: bone marrow tryptase level; ST: serum tryptase level; MT/ST ratio: bone marrow tryptase/serum tryptase ratio; SM: systemic mastocytosis; CM: cutaneous mastocytosis; CuI: cutaneous involvement; MCAS: mast cell activation syndrome

Table S3: The area under the curve for serum tryptase level, bone marrow tryptase level and MT/ST ratio in SM with CuI group versus non-CM group.

ST			MT			MT/ST ratio		
Cutpoint	Sensitivity %	Specificity %	Cutpoint	Sensitivity %	Specificity %	Cutpoint	Sensitivity %	Specificity %
≥1.2	100.0	0.0	≥1.6	100.0	0.0	≥0.8	100.0	0.0
≥3.3	100.0	7.1	≥4.2	100.0	7.1	≥0.9	100.0	7.1
≥3.5	100.0	14.2	≥4.26	100.0	14.2	≥1.0	100.0	14.2
≥4.23	100.0	21.4	≥4.5	100.0	21.4	≥1.25	100.0	21.4
≥4.24	100.0	28.5	≥4.8	100.0	28.5	≥1.28	100.0	28.5
≥4.6	100.0	35.7	≥5.8	100.0	35.7	≥1.3	100.0	42.8
≥4.9	100.0	42.8	≥7.1	100.0	42.8	≥1.3	100.0	50.0
≥5.2	100.0	50.0	≥7.8	100.0	50.0	≥1.33	100.0	57.1
≥5.4	100.0	57.1	≥11.3	100.0	57.1	≥1.6	100.0	64.2
≥5.7	100.0	64.2	≥18	100.0	64.2	≥1.7	100.0	71.4
≥5.9	97.3	64.2	≥20.8	100.0	71.4	≥1.8	97.3	71.4
≥6.2	97.3	71.4	≥21.7	100.0	78.5	≥2.2	97.3	78.5
≥6.2	94.7	71.4	≥27	97.3	78.5	≥2.3	97.3	85.7
≥8.8	92.1	71.4	≥29	97.3	85.7	≥3.8	97.3	92.8
≥10.6	89.4	71.4	≥35.4	94.7	85.7	≥4.2	94.7	92.8
≥13	86.8	71.4	≥52.8	94.7	92.8	≥4.4	92.1	92.8
≥14	84.2	71.4	≥77.4	92.1	92.8	≥4.6	89.4	92.8
≥15.9	81.5	78.5	≥99	89.4	92.8	≥4.9	86.8	92.8
≥16	81.5	85.7	≥102	86.8	92.8	≥6.6	84.2	92.8
≥18.1	81.5	92.8	≥107	84.2	92.8	≥8.2	81.5	92.8

≥ 20.4	78.9	92.8	≥ 133	81.5	92.8	≥ 9.3	78.9	92.8
≥ 21	76.3	92.8	≥ 137	78.9	92.8	≥ 9.9	76.3	92.8
≥ 21.2	73.6	100.0	≥ 158	76.3	92.8	≥ 10.1	73.6	92.8
≥ 22	71.0	100.0	≥ 190	76.3	100.0	≥ 10.1	71.0	92.8
≥ 22.9	68.4	100.0	≥ 210	71.0	100.0	≥ 10.5	68.4	92.8
≥ 23.7	65.7	100.0	≥ 216	68.4	100.0	≥ 10.7	65.7	92.8
≥ 24	63.1	100.0	≥ 245	65.7	100.0	≥ 10.9	63.1	92.8
≥ 25.1	60.5	100.0	≥ 264	63.1	100.0	≥ 11.1	60.5	92.8
≥ 25.2	57.8	100.0	≥ 274	60.5	100.0	≥ 11.4	57.8	92.8
≥ 26	55.2	100.0	≥ 331	57.8	100.0	≥ 12.2	55.2	92.8
≥ 26.4	52.6	100.0	≥ 356	55.2	100.0	≥ 15.5	52.6	92.8
≥ 27	50.0	100.0	≥ 405	52.6	100.0	≥ 17.0	50.0	92.8
≥ 29	47.3	100.0	≥ 477	50.0	100.0	≥ 18.4	47.3	92.8
≥ 31	44.7	100.0	≥ 485	47.3	100.0	≥ 19.5	44.7	92.8
≥ 32.5	42.1	100.0	≥ 496	44.7	100.0	≥ 20.2	42.1	92.8
≥ 34	39.4	100.0	≥ 665	42.1	100.0	≥ 20.4	39.4	92.8
≥ 35	36.8	100.0	≥ 716	39.4	100.0	≥ 22.0	36.8	92.8
≥ 36	31.5	100.0	≥ 777	36.8	100.0	≥ 23.6	34.2	92.8
≥ 40	28.9	100.0	≥ 912	34.2	100.0	≥ 24.2	31.5	92.8
≥ 40.1	26.3	100.0	≥ 930	31.5	100.0	≥ 25.1	28.9	92.8
≥ 46	23.6	100.0	≥ 953	28.9	100.0	≥ 25.3	26.3	92.8
≥ 46.9	21.0	100.0	≥ 1165	26.3	100.0	≥ 26.3	23.6	92.8
≥ 48	18.4	100.0	≥ 1486	23.6	100.0	≥ 26.5	23.6	100.0
≥ 61	15.7	100.0	≥ 1631	21.0	100.0	≥ 29.4	21.0	100.0
≥ 80.6	13.1	100.0	≥ 1702	18.4	100.0	≥ 39.7	18.4	100.0
≥ 88	10.5	100.0	≥ 2215	15.7	100.0	≥ 42.4	15.7	100.0
≥ 105	7.8	100.0	≥ 3242	13.1	100.0	≥ 85.5	13.1	100.0
≥ 116	5.2	100.0	≥ 3423	10.5	100.0	≥ 105.5	10.5	100.

≥ 130	2.6	100.0		≥ 4965	7.8	100.0		≥ 141.85	7.8	100.0
>130	0.0	100.0		≥ 12245	5.2	100.0		≥ 154.38	5.2	100.0
				≥ 41085	2.6	100.0		≥ 316.03	2.6	100.0
				>41085	0.0	100.0		>316.03	0.0	100.0

MT: bone marrow tryptase level; ST: serum tryptase level; MT/ST ratio: bone marrow tryptase/serum tryptase ratio; SM: systemic mastocytosis; CM: cutaneous mastocytosis; CuI: cutaneous involvement; MCAS: mast cell activation syndrome

Table S4: The area under the curve for serum tryptase level, bone marrow tryptase level and MT/ST ratio in SM without CuI group versus MCAS group.

ST			MT			MT/STratio		
Cutpoint	Sensitivity %	Specificity %	Cutpoint	Sensitivity %	Specificity %	Cutpoint	Sensitivity %	Specificity %
≥2.1	100.0	0.0	≥2.53	100.0	0.0	≥0.7	100.0	0.0
≥2.6	100.0	6.2	≥2.94	100.0	6.2	≥0.9	100.0	6.2
≥2.9	100.0	12.5	≥2.99	100.0	12.5	≥1.0	100.0	12.5
≥3.2	100.0	18.7	≥3.24	100.0	18.7	≥1.0	100.0	18.7
≥3.4	100.0	31.2	≥3.43	100.0	25.0	≥1.0	100.0	25.0
≥3.8	100.0	37.5	≥4.32	100.0	31.2	≥1.0	100.0	31.2
≥3.8	100.0	43.7	≥4.89	100.0	37.5	≥1.1	100.0	37.5
≥6.1	100.0	50.0	≥6.56	100.0	43.7	≥1.13	100.0	43.7
≥6.7	100.0	56.2	≥7.84	100.0	50.0	≥1.16	100.0	50.0
≥7.1	100.0	62.5	≥14	100.0	56.2	≥1.17	100.0	56.2
≥10.3	100.0	68.7	≥17	100.0	62.5	≥1.2	100.0	62.5
≥12	100.0	75.0	≥17.1	100.0	68.7	≥1.3	100.0	68.7
≥13.6	100.0	81.2	≥19.5	100.0	75.0	≥1.4	100.0	75.0
≥15.8	80.0	81.2	≥20.7	100.0	81.2	≥2.2	100.0	81.2
≥18	80.0	87.5	≥30.8	100.0	87.5	≥2.9	80.0	81.2
≥22	80.0	93.7	≥94	80.0	87.5	≥4.3	80.0	87.5
≥41	60.0	93.7	≥99.4	80.0	93.7	≥5.2	80.0	93.7
≥41.9	40.0	93.7	≥21	80.0	100.0	≥8.8	60.0	93.7
≥88.6	20.0	93.7	≥423	60.0	100.0	≥10.2	40.0	93.7

≥ 145	20.0	100.0		≥ 868	40.0	100.0		≥ 19.2	40.0	100.0
>145	0.0	100.0		≥ 1419	20.0	100.0		≥ 20.7	20.0	100.0
				>1419	0.0	100.0		>20.7	0.0	100.0

MT: bone marrow tryptase level; ST: serum tryptase level; MT/ST ratio: bone marrow tryptase/serum tryptase ratio; SM: systemic mastocytosis;

CM: cutaneous mastocytosis; CuI: cutaneous involvement; MCAS: mast cell activation syndrom

