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Biochemical markers predictive for bone marrow involvement in systemic mastocytosis

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ABSTRACT

Systemic mastocytosis is characterized by bone marrow involvement, which requires a bone marrow biopsy for diagnostic work-up. We questioned whether bone marrow involvement could be predicted using biochemical markers. We selected patients with various symptoms suggestive of indolent systemic mastocytosis, of whom 63 ultimately had bone marrow involvement. Patients suspected of aggressive mastocytosis, or mastocytosis associated with other hematologic diseases were excluded. Evaluation of 115 patients and 15 patient controls demonstrated a test accuracy for serum tryptase, urinary N^{τ} methylhistamine and N^{τ} methylimidazole acetic acid of 96%, 88% and 95% respectively. These markers provide an excellent pre-test probability of indolent systemic mastocytosis.

Key words: mastocytosis, bone marrow involvement, biochemical markers, tryptase, N^{τ} methylhistamine, N^{τ} methylimidazole acetic acid.

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Introduction

Mastocytosis denotes a heterogeneous group of disorders characterized by abnormal growth and accumulation of mast cells in one or more organ systems. In systemic mastocytosis the bone marrow is always involved.[1] A bone marrow biopsy with appropriate immunohistochemical studies is considered the gold standard for diagnosis. Ancillary tests such as mast cell immunophenotyping, cytogenetic/molecular studies, and serum tryptase levels assist in confirming the diagnosis.[2] While patients with aggressive systemic mastocytosis and mast cell leukemia are usually severely symptomatic, adult patients with indolent mastocytosis frequently present only with skin lesions and/or mediator-related symptoms.[3] As these patients may be reluctant to undergo a bone marrow biopsy, it might be helpful if biochemical parameters could select, with a high level of certainty, patients with and without bone marrow involvement. Analysis of biochemical mediators released by mast cells may be useful for screening and diagnostic evaluation.

We investigated the predictive value and accuracy of serum tryptase and urinary N^{τ} methylhistamine (MH) and N^{τ} methylimidazole acetic acid (MIMA) to predict bone marrow involvement in indolent systemic mastocytosis. We retrospectively analyzed blood, urine, and bone marrow data of a large cohort of adult patients who had been referred to our department with the probable diagnosis of indolent systemic mastocytosis.

Design and Methods

In 115 patients, a diagnosis of indolent systemic mastocytosis was considered because of: biopsy-proven cutaneous mastocytosis (all examined by an experienced dermatologist: adult-onset n=33; pediatric-onset n=3), unexplained anaphylactic reaction (n=6), hymenoptera anaphylaxis (n=22) or anaphylaxis in response to intravenous contrast material (n=1; the diagnosis of anaphylaxis was based on criteria as described in a recent paper by Sampson *et al.*[4]), mediator-related symptoms such as flushing, pruritus, abdominal pain, nausea, vomiting, diarrhea (n=16), unexplained severe osteo-

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Table 1. Clinical and laboratory characteristics of subjects.

	Patients (n=115)	*Controls (n=15)*
Patient characteristics		
Age (years) [median, range]	49 (16-82)	54 (20-75)
Male	41 (36%)	6 (40%)
Laboratory measurements		
Tryptase (µg/L) [median, range]	22.9 (1.0-283)	4.85 (2.3-16.5)
*Above 20 µg/L**	64 (56%)	
MH (µmol/mol creatinine) [median, range]	221 (44-2554)	68.5 (27-140)
Above 97$^{1/2th}$ percentile	85 (74%)	–
MIMA (mmol/mol creatinine) [median, range]	2.3 (0.5-50.6)	1.4 (0.8-2.4)
Above 97$^{1/2th}$ percentile	77 (67%)	1 (7%)
KIT mutation D816V*		
Positive	36 (31%)	–
Negative	50 (44%)	–
Not assessed	29 (25%)	15 (100%)
Immunophenotype* Co-expression of CD117 with CD2 and/or CD25		
Positive	39 (34%)	–
Negative	27 (23%)	–
Not assessed	49 (43%)	15 (100%

** Minor WHO criterion[12] MH: N- methylhistamine; MIMA: N- methylimidazole acetic acid.*

porosis (n=7) or combinations thereof (n=27). Patients with clinical *B* and *C* findings suggestive of aggressive mastocytosis were excluded from the study along with patients with a probable diagnosis of systemic mastocytosis with an associated hematologic non-mast cell lineage disease.[2] Fifteen patients who underwent a bone marrow biopsy for reasons not related to mast cell pathology served as controls. In these patients, bone marrow biopsies were performed for: analysis of competence as a bone marrow donor (n=1), cytopenia of any type (n=4), staging of non-hematologic malignancy (n=5), and exclusion of myeloproliferative disorder (n=3) or systemic amyloidosis (n=2). A bone marrow biopsy was performed and tryptase, MH and MIMA were determined for all patients and patient controls. The median interval (range) between bone marrow and blood/urine analysis was 0 (0-365) days. This time interval was acceptable if clinical symptoms remained unchanged because, in fact, biochemical parameters of patients with proven indolent mastocytosis are usually stable for several years.[5]

Tryptase levels were determined using the B12 assay.[6] Levels of MH were determined by an isotope-dilution mass fragmentographic method.[7] MIMA was determined as previously described[8] with some modifications using isotope dilution mass fragmentography.

Bone marrow biopsies were taken from the iliac crest by an experienced hematologist using the Jamshidi technique. The bone marrow core required a minimal length of 20 mm to be considered representative. All formalin-fixed paraffin-embedded (n=106) samples were stained using the Avidin-Biotin system for tryptase (clone AA1 from Dakocytomation, Glostrup, Denmark) and CD117 (Dakocytomation) expression. Tissue sections were stained by toluidin-blue for biopsies embedded in plastic (n=24).

For bone marrow mast cell immunophenotyping, 300,000 events were analyzed using four-color staining, including CD45, CD117, CD2 and CD25 as previously described.[9] *KIT* mutation analysis was performed as previously described.[10, 11]

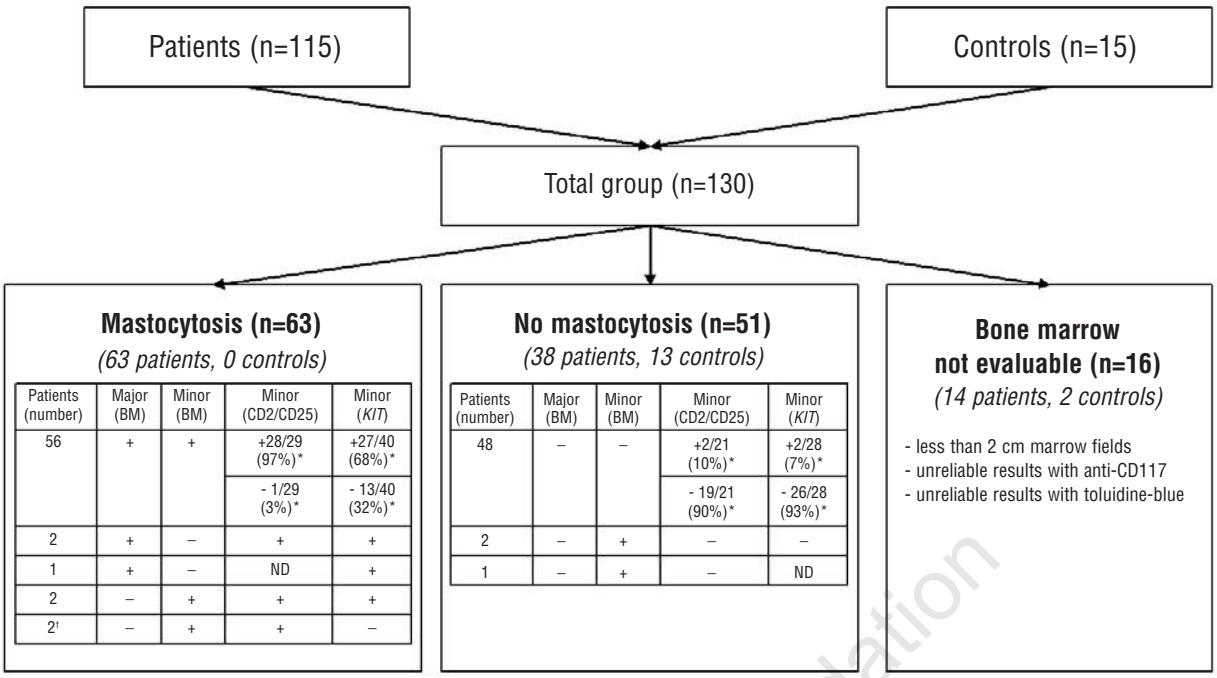
All bone marrow biopsies were revised blinded, and scored for the presence of major and minor criteria according to the WHO classification[12] by an experienced hematopathologist (PMK). Obviously, serum tryptase as a minor criterion was omitted for this specific study.

For statistical analysis, patients were grouped into positive or negative for systemic mastocytosis by outcome of bone marrow revision, the additional minor criteria CD2 and/or CD25 expression by *KIT* positive mast cells, and the D816V *KIT* mutation.[12] Receiver operator characteristic (ROC) curves were constructed with Analyse-It (Analyse-It Software, Leeds, United Kingdom) to examine the diagnostic value of each analyte. Furthermore, we analyzed whether combinations of the three biochemical markers could increase the predictive value for bone marrow involvement compared with each marker alone.

Results and Discussion

Patient characteristics of both groups were comparable (*see Table 1*). Both groups consisted of comparable subjects. Results from bone marrow histology and additional WHO minor criteria are shown in Figure 1. Fifty-nine patients showed one major and one or more minor criteria for the diagnosis of systemic mastocytosis. In 51 patients, a diagnosis of systemic mastocytosis could not be made. In this retrospective study, additional data on flow cytometry or *KIT* mutation analysis were sometimes lacking (Table I). However, we decided not to repeat these tests, either because there were already sufficient major and minor criteria for the diagnosis of systemic mastocytosis or because all other criteria were negative, indicating that no diagnosis of systemic mastocytosis could be made. Given that our study aim was to evaluate whether serum tryptase and the urinary metabolites MH and MIMA could predict systemic mastocytosis, all WHO major and minor criteria were used except serum tryptase. Consequently, 2 patients, whose diagnosis of systemic mastocytosis was based on this criterion (tryptase 23.9 and 28.9 µg/L, respectively), were excluded from further study (Figure 1).

ROC analyses were performed to study the sensitiv-



Patients (number)	Major (BM)	Minor (BM)	Minor (CD2/CD25)	Minor (*KIT*)
56	+	+	+28/29 (97%)*	+27/40 (68%)*
			- 1/29 (3%)*	- 13/40 (32%)*
2	+	–	+	+
1	+	–	ND	+
2	–	+	+	+
2†	–	+	+	–

Patients (number)	Major (BM)	Minor (BM)	Minor (CD2/CD25)	Minor (*KIT*)
48	–	–	+2/21 (10%)*	+2/28 (7%)*
			- 19/21 (90%)*	- 26/28 (93%)*
2	–	+	–	–
1	–	+	–	ND

Major (BM): Multifocal dense infiltrates of mast cells (>15 mast cells aggregating) detected in sections of bone marrow
Minor (BM): >25% of mast cells are spindle-shaped in bone marrow sections
Minor (*C-KIT*): Detection of a c-kit point mutation at codon 816 in bone marrow or blood
Minor (CD2/CD25): Kit+mast cells in bone marrow or blood co-express CD2 or/and CD25
ND: not determined
*Number of patients with present (+) and absent (-) criterion/number of patients in which criterion is determined (percentage)
†Not included: according to WHO-criteria diagnosis systemic mastocytosis (tryptase >20 ng/mL)

Figure 1. Distribution of patients according to bone marrow involvement. Distribution of subjects in indolent systemic mastocytosis/no indolent systemic mastocytosis groups used for statistical analysis, according to bone marrow pathology and evaluation of WHO minor criteria related to *KIT* mutation and CD2/CD25 immunophenotyping.

ity and specificity of serum tryptase and urinary MH and MIMA. All biochemical markers showed an area under the curve (AUC) of >80% indicating excellent test accuracy, with serum tryptase (AUC: 0.960; 95% CI: [0.930 -0.991]) and urinary MIMA (AUC: 0.948; 95% CI: [0.911 - 0.985]) performing significantly better (tryptase *vs.* MH p=0.0096, MIMA *vs.* MH p=0.0057) than urinary MH (AUC: 0.881; 95% CI: [0.823-0.940]). There was no significant difference between diagnostic accuracy of tryptase and MIMA (p=0.5613). Analysis of combinations of the three biochemical markers compared with each marker alone showed no further increase in diagnostic accuracy (*data not shown*).

Although it is not necessary to choose any particular threshold for assessment of test accuracy of the various biochemical markers, we selected various thresholds to be used in patient care (Table 2). This means that a patient with only cutaneous involvement and a serum tryptase level below <20 μg/L (threshold used by the WHO as minor criterion[12]) would have a 16% risk of bone marrow involvement, and thus a diagnosis of indolent systemic mastocytosis. This risk would further decrease to 0% if such a patient had a tryptase level <4.9 μg/L. In the absence of symptoms suggestive of mastocytosis, and given present lack of a curative therapy for systemic mastocytosis, this could be considered acceptable. On the other hand, this could help convince patients and doctors to perform a bone marrow biopsy for those with cutaneous mastocytosis or clinical symptoms suggestive of mastocytosis who have elevated levels of serum tryptase and/or urinary histamine metabolites. The probability that a patient with a serum tryptase level of ≥21.9 μg/L had bone marrow involvement was 98%, and with a level of ≥30.8 μg/L this rose even to 100%. Notably, serum tryptase is not pathognomonic for mastocytosis. Patients with high tryptase levels may harbor another disease, such as a myeloid haematologic disorder.[13]

Remarkably, clinical symptoms were helpful in predicting bone marrow involvement. Obvioulsy, this should be related to the referral pattern of this group of patients to our university hospital. The majority of the patients with anaphylactic reactions (14/24) and with cutaneous mastocytosis (21/31 evaluable patients) had systemic mastocytosis. In particular, patients with cutaneous mastocytosis with additional clinical symptoms were almost all classified as systemic mastocytosis (23/25). This agrees with the general assumption that

Table 2. Sensitivity/specificity pairs and predictive values. Sensitivity/specificity pairs and positive predictive values (PPV) and negative predictive values (NPV) of tryptase, MH and MIMA at various cut-off points.

Marker	*Cut-off point*	*Sensitivity*	*Specificity*	*PPV*	*NPV*
Tryptase	≥4.94 μg/L	100%	41.2%	67.0%	100%
	≥21.9 μg/L	83.6%	98.0%	98.4%	83.3%
	≥30.8 μg/L	67.2%	100%	100%	71.8%
MH	≥103 μmol/mol creatinine	100%	39.2%	66.3%	100%
	≥171 μmol/mol creatinine	86.9%	70.6%	77.9%	81.8%
	≥284 μmol/mol creatinine	55.7%	100%	100%	65.4%
MIMA	≥1.6 mmol/mol creatinine	100%	56.9%	73.5%	100%
	≥2.0 mmol/mol creatinine	93.4%	82.4%	86.3%	91.3%
	≥3.8 mmol/mol creatinine	49.2%	100%	100%	62.2%

adult-onset cutaneous mastocytosis commonly represents systemic mastocytosis. By contrast, 4 out of the 15 patients with mediator-related symptoms only were classified as such. The quality of the bone marrow biopsy and additional immunohistochemistry is very important, and we used strict criteria for final interpretation. Mast cell infiltrates can be sparse, therefore examination of sufficient marrow fields for a final negative diagnosis is required making additional immunohistochemistry essential. In our retrospective study, we had to discard 12% of the biopsy specimens, the majority due to small size. We used control patients instead of healthy volunteers. Besides ethical issues, the main reason for this selection was based upon the fact that the clinical question was not to identify subjects with or without bone marrow involvement in asymptomatic volunteers, but patients who are considered to have systemic mastocytosis. In conclusion, we have demonstrated in a large representative cohort of patients that blood and urinary analysis for tryptase and histamine metabolites can help to predict those patients who have bone marrow involvement representing indolent systemic mastocytosis.

Authorship and Disclosures

MLD, JJvD, FFvD, PMK, EvdV, JGRdM, IPK, HCK-N: contributed substantially to conception and design, to acquisition of data, to analysis and interpretation of data, drafted and revised the manuscript critically for important intellectual content and gave final approval of the version to be published. The authors reported no potential conflicts of interest.

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