

Tryptase and histamine metabolites as diagnostic indicators of indolent systemic mastocytosis without skin lesions

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Abstract

Background: Risk indicators of indolent systemic mastocytosis (ISM) in adults with clinical suspicion of ISM without accompanying skin lesions [urticaria pigmentosa (UP)] are lacking. This study aimed at creating a decision tree using clinical characteristics, serum tryptase, and the urinary histamine metabolites methylimidazole acetic acid (MIMA) and methylhistamine (MH) to select patients for bone marrow investigations to diagnose ISM.

Methods: Retrospective data analysis of all adults, in whom bone marrow investigations were performed to diagnose ISM, was carried out.

Results: In total, 142 patients were included. SM was absent in all 44 patients with tryptase <10 µg/l, in 45 of 98 (46%) patients with tryptase ≥ 10 µg/l and in 18 of 52 patients (35%) with tryptase >20 µg/l. Above 43 µg/l, all patients had ISM (*n* = 11). Male gender, insect venom anaphylaxis as presenting symptom, tryptase, MIMA, and MH were independent ISM predictors. If tryptase was ≥ 10 µg/l, the diagnostic accuracy of MIMA and MH was high (areas under the ROC curve 0.92).

Conclusions: In suspected patients without UP, the ISM risk is very low (if present at all) if tryptase is <10 µg/l. If tryptase is ≥ 10 µg/l, this risk depends on MIMA and MH, being low if these are normal, but high if these are elevated. Male gender and insect venom anaphylaxis are additional risk indicators. We recommend refraining from bone marrow examinations in suspected patients without UP if tryptase is <10 µg/l. Our results question the reliability of the minor diagnostic World Health Organization criterion of tryptase >20 µg/l.

Mastocytosis is characterized by a clonal proliferation of abnormal mast cells. Manifestations are because of release of mast cell mediators or mast cell infiltration in tissues. In

many adult patients, the first sign of mastocytosis is typical red to brown macules and plaques, known as urticaria pigmentosa (UP). In systemic mastocytosis (SM), mast cells accumulate in bone marrow and other extra-dermal tissues. The large majority of the adults with SM have the indolent form (ISM).

Some patients with SM lack UP (1). Without UP, a number of clinical conditions may lead to suspicion of SM, such as unexplained anaphylaxis, osteopathy (e.g. osteoporosis), neurological or constitutional symptoms, ulcerative gastrointestinal disease or chronic diarrhea, or an unexplained

Abbreviations

AUC, area under the curve; CI, confidence interval; ISM, indolent systemic mastocytosis; MH, methylhistamine; MIMA, methylimidazole acetic acid; OR, odds ratio; ROC, receiver operating characteristic; SM, systemic mastocytosis; UP, urticaria pigmentosa; WHO, World Health Organization.

'endocrinological syndrome' (2). The Year-2005 Working Conference of leading international experts in the field of mastocytosis recommends bone marrow investigation in all adults with UP to diagnose SM, irrespective of serum tryptase level (2). However, recommendations for patients with a clinical suspicion of SM without UP are lacking.

Risk indicators of ISM are especially unclear whether tryptase is ≤ 20 $\mu\text{g/l}$. A level >20 $\mu\text{g/l}$ is a minor World Health Organization (WHO) criterion for SM diagnosis (1), reflecting a high risk of SM. Data on a threshold level of tryptase, below which the probability is negligible and above which the probability on ISM is certain, are scarce and only reported from patient groups with UP and without UP taken together (3, 4).

The urinary histamine metabolites methylhistamine (MH) and methylimidazole acetic acid (MIMA) are also considered potential indicators of SM risk (5, 6). In a previous study, we found no difference between the diagnostic accuracy of tryptase and MIMA. Combinations of tryptase, MH, and MIMA did not further increase the diagnostic accuracy (4). However, it is uncertain whether tryptase and MIMA are interchangeable with respect to their predictive potential in the individual patient, as no relation was found between 24-hour MIMA excretion and tryptase levels (7).

This study aimed at creating a decision tree using clinical characteristics, tryptase, MH, and MIMA to select patients for bone marrow investigations to establish the diagnosis of ISM.

Materials and methods

Patients

All data collected in our institute from 1981 till May 2011 concerning adult patients who underwent diagnostic bone marrow procedures to establish the diagnosis of SM were analyzed. In general, these procedures were performed based on clinical suspicion on SM, in most cases combined with a tryptase level >11.4 $\mu\text{g/l}$ (the upper 95th percentile in an apparently healthy population), and/or MH excretion >154 $\mu\text{mol/mol}$ creatinine and/or MIMA excretion >1.9 mmol/mol creatinine.

The diagnosis of SM was established according to the WHO criteria (1). SM was ruled out if histology and cytology did not reveal the major criterion nor the minor criterion of an abnormal morphology of $>25\%$ of the mast cells in a bone marrow biopsy of adequate quality. In case of a biopsy of inadequate quality, SM was ruled out if all minor criteria except for the criterion of serum tryptase >20 $\mu\text{g/l}$ were negative.

Patients with clinical B and C findings [with exception of those with osteoporotic fractures (8)] suggestive of smoldering and aggressive SM, respectively, were excluded along with patients with a probable diagnosis of SM with an associated hematological non-mast cell lineage disease (1). Other exclusion criteria were as follows: (a) the presence of UP (also UP in remission), (b) missing data on serum tryptase, (c) lack of biochemical, cytological, or histological data from

bone marrow to exclude the possibility of SM with certainty, and (d) renal failure (serum creatinine >120 μM) or hemodialysis. The absence of UP was established by the attending physician in SM-negative patients and by an experienced dermatologist (PCvVV) in patients with ISM. Applying the above-mentioned procedures, 89 patients without SM and 53 patients with ISM could be included (Fig. 1).

The results were used of the tryptase measurements with the shortest time interval to the bone marrow biopsy and of the MH and MIMA determinations with the shortest interval from the tryptase sampling. A time interval of >1 year was considered to be acceptable if the clinical symptoms remained unchanged. In the group without SM, tryptase sampling has been performed at the same day of the bone marrow biopsy in all but 12 patients. In only one patient, the interval was >1 year (421 days). Tryptase and histamine metabolite sampling took place at the same day except for eight patients, but in all patients within 1 year. In the ISM group, tryptase sampling has been performed at the same day of the bone marrow biopsy in all but 19 patients. In six patients, the interval was >1 year (maximal interval 2799 days). This long interval between tryptase sampling and bone marrow biopsy is because of the fact that data on bone biopsies were available from 1981 and on tryptase only from 1997. Tryptase and histamine metabolite sampling did not take place at the same day in ten patients for MH and in 11 patients for MIMA. The interval was >1 year in two and one patients (both maximal 371 days) for MH and MIMA, respectively.

The Medical Ethical Review Board of the University Medical Center Groningen declared that the study has been carried out in accordance with regulations of the review board for publication of patient data.

Biochemical markers

Tryptase assay

Serum tryptase levels were determined with the B12 assay (9), using ImmunoCAP Tryptase reagents and the Phadia 250 analysis device (Phadia AB, Uppsala, Sweden). Reference

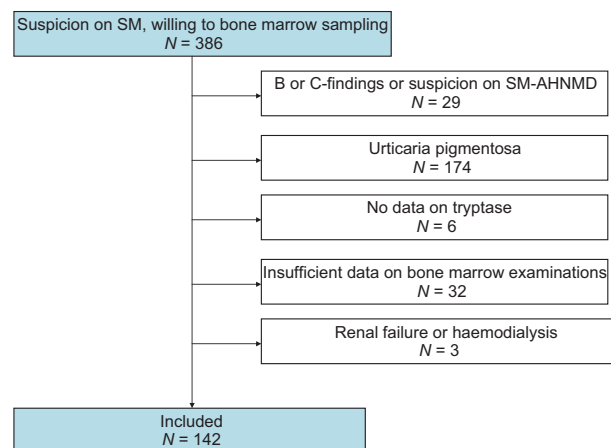


Figure 1 Flowchart of the patient selection process.

values for healthy individuals are those reported by Phadia, showing a geometric mean level of 3.8 µg/l and an upper 95th percentile of 11.4 µg/l. The inter-assay analytical coefficient of variation in our laboratory is 5.8%. Tryptase concentrations >10 µg/l were verified for interference by heterophilic antibodies (10). These antibodies were found in three SM-negative cases, and the corrected tryptase concentrations were used.

Histamine metabolite assays

To measure MH and MIMA, urine samples were collected after an overnight fast, discarding the first voiding after waking. During 24 h before urine collection, patients were asked refraining from histamine-rich foods and drinks, such as sauerkraut, canned fish, yoghurt, and wine. Levels of MH were determined by an isotope-dilution mass fragmentographic method (11–13). MIMA was determined as described previously (14), with some modifications using isotope-dilution mass fragmentography. The normal values for MH and MIMA excretion in urine collected after overnight fast have been established in a previous study on an apparently healthy population (19 men, 17 women; ages 13–61 years) without performing bone marrow investigations (15). The mean ± SD values (ranges) are 101 ± 33 (50–154) µmol/mol creatinine and 1.3 ± 0.3 (0.9–1.9) mmol/mol creatinine, respectively. The inter-assay analytical coefficient of variation in our laboratory is 6.8% for MH and 4.2% for MIMA.

Bone marrow samples

Bone marrow biopsies were taken from the iliac crest by an experienced hematologist according to the Jamshidi technique. To classify a bone marrow trephine biopsy negative for the presence of the histological major criterion of multifocal clusters or cohesive aggregates/infiltrates of >15 mast cells, a minimum length of 20-mm bone marrow had to be examined. In case of any doubt, multiple deeper sections were made. Formalin-fixed paraffin-embedded bone marrow samples were stained by hematoxylin and eosin, and Giemsa. Immunohistochemistry for tryptase and CD117 was performed on 3-µm-thick tissue sections using a Nexes or Benchmark Ultra automated immunostainer (Ventana Medical Systems, Tucson, AZ, USA). Primary antibodies used were anti-mast cell tryptase, clone AA1 (Dakocytomation, Glostrup, Denmark), and affinity-isolated polyclonal rabbit anti-human CD117 (Dakocytomation). All bone marrow biopsies were revised by an experienced hemato-pathologist (PMK).

Bone marrow aspirates were recovered in EDTA, and smears were stained for May-Grunwald-Giemsa and toluidine-blue. Mast cells were analyzed outside the marrow particles, and atypical morphology was recorded.

Immunophenotyping

For bone marrow mast cell immunophenotyping, 300 000 events were analyzed using four-color staining with CD45-peridinin-chlorophyll protein/cyanine 5.5, CD117-allophycocyanine, CD2-phycoerythrin, and CD25-fluorescein isothiocyanate (all

derived from Becton Dickinson Biosciences, San Jose, CA, USA). Expression of CD2 and CD25 was measured on CD45-positive/bright CD117-positive mast cells with the isotype pattern used as control. The results were analyzed on a FacsCalibur flow cytometer (Becton Dickinson Biosciences) using WINLIST 5.0 software (Verity Software House, Inc.; www.vsh.com). No mast cells were detectable (detection limit 0.01%) in the bone marrow aspirate of three SM-negative patients.

C-KIT mutation analysis

To detect the KIT D816V mutation, RNA was initially isolated from EDTA-anticoagulated bone marrow cells with the QIAamp® RNA Blood MINI Kit (QIAGEN, Westburg, Leusden, the Netherlands). C-DNA was synthesized using the Promega Reverse Transcriptase kit (Promega Benelux, Leiden, the Netherlands) and amplified using previously described primers (16, 17). The resulting 346-bp PCR product was digested with Hae III and Hinf I (BioLabs, Westburg, Leusden, the Netherlands) to detect the wild-type and the D816V mutation by agarose gel electrophoresis. From December 2007, detection of the KIT-D816V mutation was performed with a real-time PCR using previously described primers 5'-TTGTGATTTTGGTCTAGCCAGACT-3' and 5'-GTGCCATCCACTTCACAGGTAG-3' (18).

Protocol

A protocol was made to create a decision tree applicable in patients suspected to have ISM without UP. This protocol consisted of the following stepwise procedures: (a) determining which are clinical- and biochemical-independent predictors of the diagnosis of ISM; (b) if tryptase appeared to be an independent predictor, determining which is the highest level with 100% sensitivity and the lowest level with 100% specificity; and (c) if one of the histamine metabolites appeared to be an independent predictor, constructing receiver operating characteristic (ROC) curves in the total group and the group of patients with a tryptase level above the highest 100% sensitivity level.

Statistical methods

Statistical analysis was performed with PASW Statistics 18 (SPSS, Chicago, IL, USA) and Analyse-It version 2.20 (Analyse-It Software, Leeds, UK). Patients were grouped into positive or negative for ISM by the outcome of bone marrow histology and the additional minor criteria according to the WHO criteria except for the criterion of serum tryptase >20 µg/l. Results were expressed as mean ± SD or median (interquartile range) for normally distributed and non-normally distributed data, respectively. Group differences were tested using the independent samples *t*-test and Mann-Whitney *U*-test. Percentages between groups were compared using the Chi-square test and Fisher's exact test. Spearman's correlation coefficients were used to analyze interrelations between tryptase, MIMA, and MH values. Predictor analysis of ISM was performed using univariate and

Table 1 Clinical and biochemical characteristics of the patients without systemic mastocytosis vs the patients with indolent systemic mastocytosis without urticaria pigmentosa

Item	No SM	ISM	P-value
Patients, <i>n</i>	89	53	
Male,* <i>n</i> (%)	22 (25)	25 (48)	0.006
Age (years)	49.3 ± 13.7	53.1 ± 10.7	0.081
Tryptase (µg/l)	12.2 (4.28–18.3)	24.6 (16.7–39.0)	<0.0001
MH (µmol/mol creat)	120 (82–183)	231 (185–339)	<0.0001
MIMA (mmol/mol creat)	1.6 (1.3–2.0)	2.8 (2.3–4.2)	<0.0001
CD2 expression positive, <i>n/n</i> (%)	0/76 (0)	28/33 (85)	<0.0001
CD25 expression positive, <i>n/n</i> (%)	0/76 (0)	32/33 (97)	<0.0001
D816V mutation positive in bone marrow aspirate, <i>n/n</i> (%)	0/86 (0)	33/39 (85)	<0.0001
D816V mutation positive in peripheral blood cells, [†] <i>n/n</i> (%)	0/1 (0)	1/13 (8)	1.000
Presenting symptoms			
Insect venom anaphylaxis, <i>n/n</i> (%)	19/89 (21)	30/53 (57)	<0.0001
Any anaphylaxis, <i>n/n</i> (%)	30/89 (34)	36/53 (68)	<0.0001
Flushing, <i>n/n</i> (%)	21/89 (24)	6/53 (11)	0.081
Bone disease, <i>n/n</i> (%)	13/89 (15)	6/53 (11)	0.622
Other, <i>n/n</i> (%)	25/89 (28)	5/53 (9)	0.010
Creatinine (µM)	73 (64–84)	74 (66–87)	0.343

Values are mean ± SD or median (interquartile range) unless otherwise indicated.

SM, systemic mastocytosis; ISM, indolent systemic mastocytosis; MH, methylhistamine; MIMA, methylimidazole acetic acid.

*In one patient, establishment of gender was impossible because the diagnosis of ISM was made after a gender transformation procedure.

[†]In those patients in whom D816V mutation analysis has not been performed in bone marrow aspirate.

multivariate logistic regression with conditional stepwise forward inclusion of variables that had a *P*-value ≤ 0.30 in univariate analysis. The probability of *P* for stepwise entry was 0.05. ROC analysis was performed to examine the diagnostic value of each analyte (19). Areas under the curves (AUCs) and their 95% confidence intervals (CI) were evaluated as measures of diagnostic accuracy (20). Area under the curve >0.90 was interpreted as high accuracy (21). Furthermore, sensitivity and specificity of tryptase, MH, and MIMA were assessed at several cut-off points. *P*-value <0.05 was considered to be statistically significant.

Results

A total of 142 patients without UP were included. An overview of the clinical and biochemical characteristics is given in Table 1. SM was absent in all 44 patients with tryptase <10 µg/l, in 45 of 98 patients (46%) with tryptase ≥ 10 µg/l and in 18 of 52 patients (35%) with tryptase >20 µg/l. Above 43 µg/l, all patients had ISM (*n* = 11). In one SM-negative patient, neither C-KIT mutation analysis nor immunophenotyping has been performed. In all SM-negative patients with tryptase >20 µg/l, the major and at least two minor diagnostic criteria other than that of tryptase >20 µg/l were absent. The presenting symptom category 'other' consists of a large variety of symptoms and signs. In the SM-negative group, there were a larger number of patients with urticaria, unspecified skin disease and/or itching, and eosinophilia than in the ISM group (data not shown).

The Spearman rank correlation coefficients are between tryptase and MH 0.265 (*P* = 0.002), between tryptase and

MIMA 0.355 (*P* < 0.0001), and between MH and MIMA 0.746 (*P* < 0.0001). Box plots and scatter diagrams are shown in Fig. 2. Predictor analysis was performed to identify parameters related to ISM. Male gender, insect venom anaphylaxis, and any anaphylaxis as presenting symptom, tryptase, MH, and MIMA were associated with a higher ISM risk in univariate regression analysis. Multivariate regression analysis showed that male gender, insect venom anaphylaxis as presenting symptom, tryptase, and MIMA were independently related to ISM risk (Table 2). MH was not selected during forward conditional logistic regression, because of the strong correlation with MIMA (*ρ* = 0.745, *P* < 0.00001). It is noteworthy, that higher MH was also independently related to ISM risk in the presence of tryptase, gender, insect venom anaphylaxis, and symptoms other than anaphylaxis or bone disease (odds ratio 1.017, 95% CI 1.009–1.025, *P* < 0.0001), indicating that MIMA as well as MH are important. The Nagelkerke *R*² of the multivariate models including MIMA and MH were 0.751 and 0.701, respectively.

The ROC curves for tryptase, MIMA, and MH to discriminate between SM-negative patients and patients with ISM are shown in Fig. 3. The 100% sensitivity cut-off level of tryptase was 10 µg/l and that of 100% specificity 43 µg/l. Area under the curves with 95% CI are for tryptase 0.84 (0.78–0.91), for MH 0.83 (0.76–0.90), and for MIMA 0.87 (0.81–0.93).

Omitting all patients with a tryptase <10 µg/l, AUCs were for MIMA and MH 0.92 (95% CI 0.87–0.97) and 0.92 (95% CI 0.87–0.98), respectively. The level with the highest combination of sensitivity and specificity was

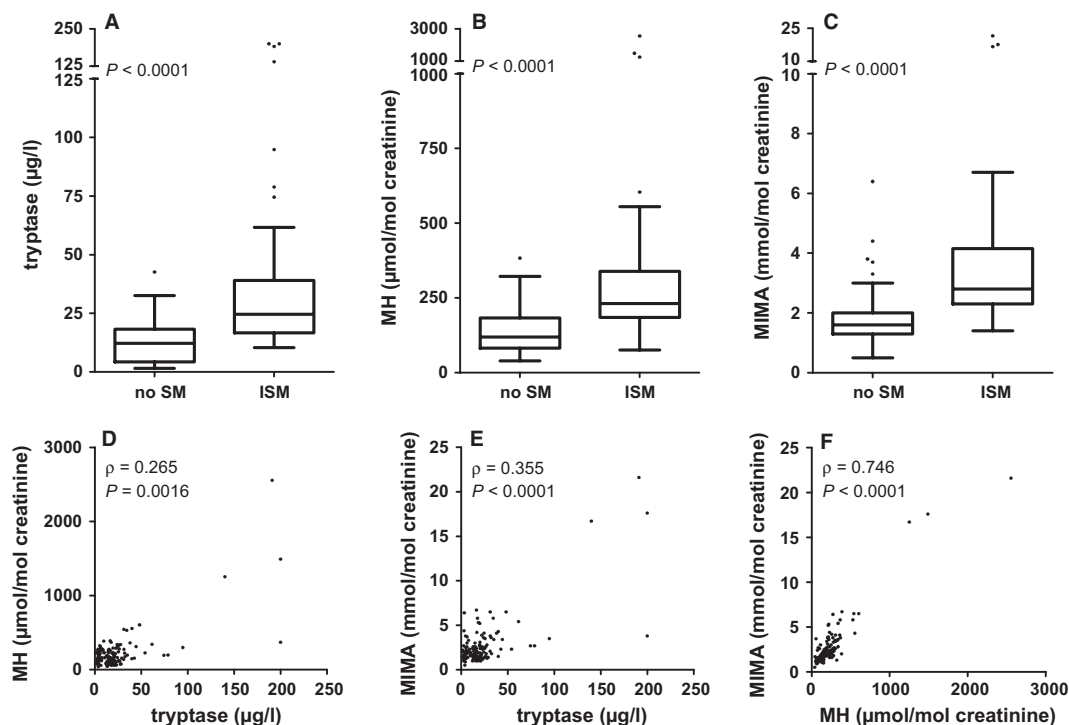


Figure 2 Box plots A, B, and C of tryptase, methylhistamine (MH), and methylimidazole acetic acid (MIMA) values, respectively, in patients without systemic mastocytosis (no SM) and with indolent systemic mastocytosis (ISM), and scatter plots D, E, and F of the relations between tryptase, MH, and MIMA. Boxes indicate medians with interquartile ranges; whiskers indicate 1.5 times the interquartile distances; • indicate outliers

Table 2 Results of univariate and multivariate logistic regression analysis for risk of indolent systemic mastocytosis without urticaria pigmentosa

	Univariate analysis		Multivariate analysis	
	OR (95% CI)	P-value	OR (95% CI)	P-value
Gender*	2.820 (1.364–5.831)	0.005	4.853 (1.389–16.958)	0.013
Age (years)†	1.025 (0.997–1.054)	0.083	—‡	—‡
Tryptase (µg/l)§	1.152 (1.091–1.216)	0.000	1.177 (1.081–1.281)	0.000
MH (µmol/mol creatinine)§	1.014 (1.009–1.019)	0.000	—‡	—‡
MIMA (mmol/mol creatinine)§	3.719 (2.223–6.221)	0.000	4.848 (2.309–10.181)	0.000
Presenting symptom¶				
Insect venom anaphylaxis	4.805 (2.286–10.103)	0.000	10.833 (2.932–40.029)	0.000
Any anaphylaxis	4.165 (2.017–8.600)	0.000	—‡	—‡
Flushing	0.413 (0.155–1.102)	0.077	—‡	—‡
Bone disease	0.746 (0.266–2.098)	0.579	—**	—**
Other	0.267 (0.095–0.747)	0.012	—‡	—‡
Creatinine§	1.011 (0.986–1.037)	0.371	—**	—**

See Table 1 for abbreviations.

OR refers to the risk of indolent systemic mastocytosis without urticaria pigmentosa;

*If gender is male.

†Per year.

‡The variable was not selected during multivariate regression analysis.

§Per grade (1 µg/l for tryptase; 1 µmol/mol creatinine for MH, 1 mmol/mol creatinine for MIMA, and 1 µM for creatinine).

¶If presenting symptom is present.

**The variable was not tested in multivariate regression analysis because of a P-value > 0.30.

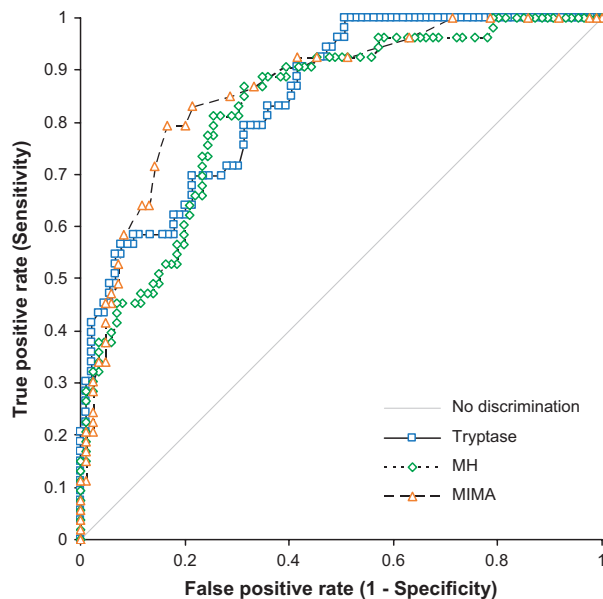


Figure 3 ROC curves for serum tryptase, urinary methylhistamine (MH), and urinary methylimidazole acetic acid (MIMA) to discriminate between patients with indolent systemic mastocytosis and without systemic mastocytosis. Area under the curve with 95% confidence interval is for tryptase 0.84 (0.78–0.91), for MH 0.83 (0.76–0.90) and for MIMA 0.87 (0.81–0.93).

2.0 mmol/mol creatinine for MIMA (sensitivity 0.85 and specificity 0.86) and 176 $\mu\text{mol/mol}$ creatinine for MH (sensitivity 0.81 and specificity 0.93). Applying these cut-off values in the patients with tryptase $>10 \mu\text{g/l}$, MIMA levels $\geq 2.0 \text{ mmol/mol}$ creatinine were found in six of 43 SM-negative patients (two missing values) and in 45 of the 53 patients with ISM. MH levels $\geq 176 \mu\text{mol/mol}$ creatinine were found in three of 44 SM-negative patients (one missing value) and in 43 of the 53 patients with ISM. Combining both cut-off levels, seven of 43 SM-negative patients and 47 of the 53 patients with ISM had an elevated MH and/or MIMA level.

Discussion

This study shows that the risk of ISM is very low (if present at all) in patients with clinical suspicion on SM, but without UP if serum tryptase is $<10 \mu\text{g/l}$. If serum tryptase level is higher, the ISM risk depends on the urinary excretions of MH and MIMA, being low if these are normal, but high if these are elevated (MH $>176 \mu\text{mol/mol}$ creatinine and MIMA $>1.9 \text{ mmol/mol}$ creatinine). Male gender and insect venom anaphylaxis as presenting symptom are additional risk indicators. These results suggest that bone marrow examinations are not indicated in suspected patients if tryptase is $<10 \mu\text{g/l}$. In many patients in this tryptase category, the histamine metabolite excretion was elevated. This is most likely explained by a selection bias. Histamine metabolite excretion was routinely tested in our hospital in all patients with symptoms suggestive of SM, irrespective of tryptase measure-

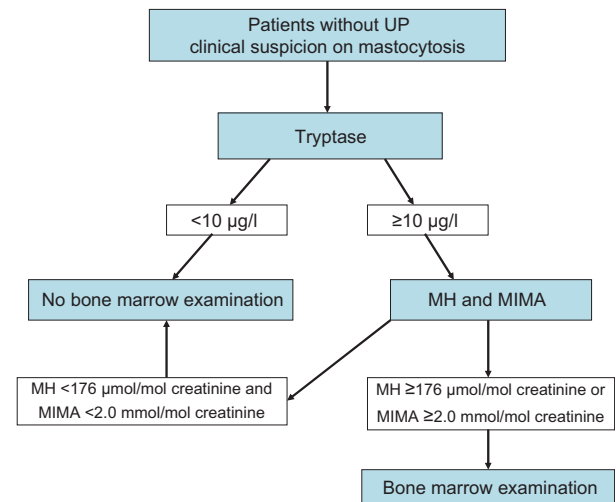


Figure 4 Decision tree to select patients for bone marrow examination to establish the diagnosis of indolent systemic mastocytosis. UP, urticaria pigmentosa; MH, methylhistamine; MIMA, methylimidazole acetic acid.

ments. In case of a low tryptase level, the decision of the attending physician to perform bone marrow examinations to exclude SM has often been taken because of an increased histamine metabolite excretion. The present analysis shows that even in this group of patients, bone marrow examinations do not seem warranted if tryptase is $<10 \mu\text{g/l}$. The decision tree to select patients for bone marrow investigations to diagnose ISM is shown in Fig. 4.

Our findings are particularly relevant because a recent study showed that the risk of anaphylactic reactions on insect stings increases gradually with increasing tryptase levels $>5 \mu\text{g/l}$ (22). This might be interpreted as a reason to use this tryptase level as threshold to start SM diagnostics. Our study demonstrates, however, that this elevated risk of anaphylactic reactions on insect stings is not because of underlying SM in patients without UP if tryptase level is $<10 \mu\text{g/l}$.

Our study also demonstrates that a substantial number of the patients in whom underlying SM with bone marrow involvement was excluded had (unexplained) tryptase levels $>20 \mu\text{g/l}$ and normal histamine metabolite excretions. Although just a minor criterion, this finding questions the reliability of the WHO criterion of a serum tryptase level $>20 \mu\text{g/l}$ for the diagnosis of SM. This criterion is supposed to indicate indirect evidence of an increased mast cell stimulation and increased mast cell mass because of the 'gain-of-function' mutation at the 816 position of the *C-KIT* proto-oncogene (23), as generally found in nearly all patients with ISM (24). Omitting this criterion, the ISM diagnosis could not have been established in three of the 53 patients with ISM. Myeloid neoplasms are sometimes accompanied by elevated tryptase levels (1), but were excluded in all patients.

The reason why some SM-negative patients had an elevated histamine metabolite excretion notwithstanding a normal tryptase level and the absence of a myeloproliferative

disorder (25) is a matter of speculation. The amount of MH and MIMA produced does not solely depend on histamine synthesis and subsequent release, but also on the contribution of exogenous histamine from food and drinks (26, 27), and on the activity of the enzymes histamine *N*-methyltransferase and diamine oxidase, which break down histamine into methylhistamine, methylimidazole acetic acid, and imidazole acetic acid (27). The latter metabolite is also a degradation product of histidine and has therefore not been measured. Genetic polymorphisms (28), alcohol (27, 29, 30), and drugs (27) can influence these enzyme activities, potentially leading to changes in the distribution of the three excreted metabolites. To minimize the contribution of exogenous histamine, we asked the patients to refrain from histamine-rich foods and drinks during the 24 h before urine sampling.

In summary, in suspected patients without UP, ISM risk is very low if serum tryptase is $<10 \mu\text{g/l}$ and depends on histamine metabolite excretion if serum tryptase is $>10 \mu\text{g/l}$. We recommend therefore refraining from bone marrow examinations in these patients if serum tryptase is $<10 \mu\text{g/l}$.

Author contributions

J.J. van Doormaal did the acquisition of the data of all patients, created the design of the study, and wrote the

paper. E. van der Veer participated in the design of the study, performed the statistical analysis, and critically revised the manuscript. P.C. van Voorst Vader did the dermatological examinations and critically revised the manuscript. P. M. Kluin did the blinded revision of all bone marrow biopsies and critically revised the manuscript. A.B. Mulder collected the data on bone marrow smears, immunophenotyping, and C-KIT mutation analyses and critically revised the manuscript. S. van der Heide performed the analyses for interference by heterophilic antibodies in the tryptase determinations and critically revised the manuscript. S. Arends performed the statistical analysis and interpretation of data and drafted the manuscript. J.C. Kluin-Nelemans, J.N.G. Oude Elberink, J.G.R. de Monchy participated in the design of the study and critically revised the manuscript.

Conflicts of interest

None for all authors.

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