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Assessment of *in vivo* mast cell reactivity in patients with systemic mastocytosis

Running head: Mast cell reactivity in patients with mastocytosis

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Abstract (words 291)

Background

Patients with systemic mastocytosis (SM) have clinical signs of mast cell (MC) activation and increased levels of MC mediators. It is unclear whether the increased mediator levels are caused by increased numbers of tissue MCs, or whether these cells in affected individuals have a hyperactive phenotype.

Objective

To determine reactivity of the skin and the airways to directly acting mediators and indirectly acting mast cell secretagogues in subjects with SM.

Methods

Skin reactivity to morphine and histamine, and airway responsiveness to mannitol and methacholine was assessed in 15 patients with SM, 11 patients with allergic asthma (A) and 13 healthy controls (HC). Serum tryptase and urinary metabolites of the MC mediators histamine and prostaglandin D₂ were measured, as well as *ex vivo* basophil histamine release.

Results

Mast cell mediators in the blood and urine were significantly higher in patients with SM than in HC and A controls. Responsiveness to local activation of skin MCs (by morphine) and airway MCs (by mannitol) was similar in SM and HC groups. Likewise, end-organ responsiveness in the skin to histamine, and in the airways to methacholine, was similar in all three subject groups. There was no evidence of increased basophil reactivity in SM patients.

Conclusions & Clinical Relevance

Mast cells in the skin and airways of subjects with SM do not exhibit hyper-reactivity towards the MC-activating stimuli morphine and mannitol, respectively. Therefore, the highly elevated baseline levels of MC mediators in SM are most likely due to increased MC numbers, rather than altered MC responsiveness. The underlying mechanisms could involve leakage of MC mediators, or dysfunctions in mediator synthesis, storage and release. One clinical implication of our study is that there is no contraindication to perform skin tests using morphine in subjects with mastocytosis.

Key words: mast cell reactivity, mastocytosis, histamine, mannitol, tryptase, prostaglandin D₂

Introduction

Upon activation, mast cells (MCs) release pre-formed and newly synthesized mediators including histamine, proteases, proteoglycans, eicosanoids and cytokines, such as TNF- α (1, 2). Together, these mediators are responsible for many of the acute signs and symptoms of mast cell-mediated reactions (1).

Mastocytosis is a clonal mast cell disorder characterized by activation and accumulation of these cells in the skin and/or internal organs (3). By definition, patients with pathologic MCs limited to the skin will be classified as having cutaneous mastocytosis, whereas patients with systemic mastocytosis (SM) have pathologic MCs in the bone marrow as well as several extra-cutaneous tissues (3). The most common variant of SM is indolent systemic mastocytosis (ISM), with or without cutaneous involvement (4). The clinical symptoms observed in mastocytosis, such as flushing, pruritus, palpitations, hypotension, syncope, abdominal pain, diarrhea, headache, fatigue, are mainly attributed to local or systemic MC-mediator release (4).

Patients with SM typically display elevated levels of MC mediators such as tryptase (5), histamine (6, 7) and urinary metabolites of prostaglandin (PG) D₂ (8), and exhibit a “clinical state of hyper-reactivity” with recurrent mediator-related symptoms including anaphylaxis (4, 9). Interestingly, most SM patients experience a waxing and waning disease course, with periods of symptom exacerbations and periods of clinical quiescence, despite chronically increased levels of mast cell mediators.

In the present study, we hypothesized that patients with SM might display altered reactivity towards challenges that activate tissue MCs. A previous study on SM patients reported no apparent change in skin reactivity (10), but airway responsiveness in SM has not yet been explored. We therefore assessed tissue responsiveness to MC-activating stimuli, using morphine in the skin and mannitol in the airways. We also examined end-organ responsiveness to histamine in the skin and methacholine in the airways. To assess whole-body MC activation, we measured baseline serum levels of tryptase, and urinary levels of the major histamine metabolite (1-methyl-4-imidazoleacetic acid; i.e., *tele*-MIAA) and the PGD₂ metabolite (11β – PGF_{2α}). These mediators are clinically the most relevant and feasible to assay. Histamine release from basophils was also explored as a possible indicator of general disturbances in mediator secretion.

Methods

Study subjects and design

In this study, 15 cases (≥18 years) with systemic mastocytosis (5 men and 10 women), with a median age of 48 years (21 to 69), were recruited between April 2010 and March 2013 from the outpatient clinic of Respiratory Medicine and Allergy at Karolinska University Hospital Huddinge. All investigated subjects were in a clinically stable period of their condition during the visit. Exclusion criteria were usage of beta blockers, inability or refusal to undergo a bone marrow biopsy and aspirate, HIV or other known immunodeficiency, carcinoid syndrome, pheocromocytoma or pregnancy. The Ethical Review Board in Stockholm approved the study (Dnr 2009/959-31-4, 2009/1422-32), and subjects provided written informed consent.

Two control groups were recruited for comparisons of mast cell mediators, skin tests, and *ex vivo* basophil histamine release. One group consisted of 13 healthy volunteers (eight women, five men), aged 22 to 55 years with no history of allergic diseases. The other group included 11 subjects (eight men, three women) aged 22 to 52 years with mild allergic asthma treated with short-acting beta-agonists only. The results of the bronchial challenges were compared with established reference values.

Subjects underwent a complete clinical evaluation including physical examination and history, routine biochemistry and skin prick testing. Baseline pulmonary function testing and exhaled nitric oxide (FeNO) measurements were also performed, and airway responsiveness was assessed by methacholine inhalation challenge. Serum and urine samples were collected for analyses of baseline serum tryptase, *tele*-MIAA and 11β – PGF_{2α}. At a separate visit, a bronchial challenge with mannitol was performed to assess bronchial MC reactivity in patients with SM.

Diagnostic criteria

The diagnosis of SM was made using current WHO criteria, and based on bone marrow investigations to confirm the presence/absence of major and/or minor criteria (3). All SM patients obtained a final diagnosis of indolent systemic mastocytosis (ISM) after staging investigations by computerized tomography and bone mineral density to exclude other variants of SM such as SM-associated hematologic non-MC-lineage disease, or aggressive systemic mastocytosis.

Anaphylactic reactions were diagnosed according to NIH clinical criteria (11), when either reduced blood pressure or associated-symptoms like syncope/pre-syncope; and/or respiratory compromise or laryngeal oedema were present accompanied by the involvement of skin-mucosal tissue or gastrointestinal symptoms. Patients with recurrent, unexplained anaphylaxis underwent an extensive work-up to exclude other causes of anaphylaxis before obtaining a diagnosis of idiopathic anaphylaxis. Hereditary angioedema, pheochromocytoma, and carcinoid tumors were ruled out using appropriate investigations.

Atopic status was defined by at least one positive SPT reaction and/or a positive aeroallergen CAP. Atopic subjects suffering attacks of dyspnea or wheezing upon contact with a particular allergen were considered to have allergic asthma. Subjects received an asthma diagnosis according to GINA criteria (12), i.e. typical clinical symptoms accompanied with documented airway reversibility (>12% and 200ml) or increased airway responsiveness following a bronchial challenge test.

Allergy work-up

Skin prick testing (SPT) was performed using commercial extracts (ALK Allergologisk Laboratorium A/S, Horsholm, Denmark) of standard aeroallergens (birch, timothy grass and mugwort pollens, cat, dog and horse dander, house dust mites, moulds) and food allergens (milk, egg, nuts, cereals, codfish, shrimp), and hymenoptera venom (bee and wasp). All subjects had abstained from antihistamines for at least 72 hours before SPT. Additionally; SPT using morphine (10mg/ml), a MC secretagogue was performed. Controls used were histamine dihydrochloride (10mg/ml) and saline (NaCl 0.9%). In accordance with EAACI guidelines (13), a skin prick test was considered positive if the wheal diameter was at least 3 mm larger than that elicited by the saline control. The wheal size was measured after 15 minutes and recorded as the mean diameter (adding the longest diameter to the orthogonal diameter and dividing it by 2).

Serum concentrations of IgE (kE/L) were determined in all subjects (ImmunoCAP[®] Total IgE, ThermoFisher, Uppsala, Sweden). Specific IgE tests (ImmunoCAP[®] Phadiatop[®], ThermoFisher) were performed in six of the cases (four patients with venom-induced anaphylaxis, one unexplained anaphylaxis patient with dermatographism and one UP patient who was not able to abstain from antihistamines).

Measurement of Mast Cell Mediators

One blood and two urine samples were collected on the same study day. Samples were stored at -70°C until analysed. Serum tryptase levels were measured using the ImmunoCAP[®] tryptase assay (ThermoFisher). The major urinary histamine metabolite *tele*-MIAA was measured by LC/MS (14) and values were expressed as micromole per milimole creatinine. The early prostaglandin D₂ metabolite, 11β-PGF_{2α}, was measured using an in-house enzyme immunoassay method with commercially available reagents (Cayman Chemical Co., Inc., Ann Arbor, MI) (15). Absolute values were expressed as nanograms per milimole creatinine. Urinary creatinine concentrations were measured by automated colorimetric Jaffe method.

Measurement of pulmonary function and exhaled nitric oxide

Pulmonary function was measured using a dynamic spirometer (Jaeger MasterScope, software version 5.31; Intramedic AB, Sollentuna, Sweden).

The fraction of exhaled nitric oxide (FeNO) was measured prior to spirometry using a NIOX analyser (Aerocrine AB, Solna, Sweden), at a flow rate of 50 mL/s according to American Thoracic Society guidelines (16).

Methacholine provocation test

Methacholine (MCh) (prepared at Karolinska University Hospital Pharmacy, Stockholm, Sweden) inhalation challenges were performed using a dosimeter-controlled jet nebulizer (Spira Electro 2; Respiratory Care Center, Hämeenlinna, Finland) (17), and began with the inhalation of saline. Provided the FEV₁ did not change by more than 10%, inhalation of methacholine was commenced with the post-diluent FEV₁ value used as baseline. Doubling doses of methacholine were inhaled every third minute, starting with 14.2 µg. The challenge was terminated when the subject's FEV₁ dropped by 20% (PD₂₀) or more, or when the highest cumulated dose of methacholine (7256 µg) had been attained. Reference values have been defined previously (18), with a cut-off value corresponding to the 15th percentile of the distribution of healthy individuals.

Mannitol provocation test

Mannitol (Aridol, Pharmaxis, Frenchs forest, NSW, Australia) capsules were inhaled using a dry powder inhaler (Plastiapne, Osnago, Italy). The challenge was initiated with an empty capsule and FEV₁ was measured in duplicates 60s later. If the fall in FEV₁ was <10% of baseline, challenge proceeded, commencing with 5 mg of mannitol. Spirometry was performed 60s later and if the fall in FEV₁ was < 15%, the dose of mannitol was increased stepwise (10, 20, 40, 80, 160, 160, 160 mg) until the fall in FEV₁ was >15% or the maximum cumulative dose (635 mg) according to the protocol had been administered (19).

Basophil histamine release assay

Five ml of heparinized whole blood was collected for the basophil histamine release assay (RefLab Aps, Copenhagen, Denmark). Briefly, blood was washed with piperazine-N,N'-bis (2-ethanesulfonic acid) (PIPES) buffer and incubated on the assay plate for 60 minutes at 37°C with increasing concentrations of anti-human IgE (0.01, 0.03, 0.1, 0.4, 1.4 and 5 µg/ml).

Statistical analysis

Statistical analyses were performed using GraphPad Prism 5, and differences were considered significant if $p < 0.05$. Categorical variables are presented as numbers and percentages and were compared using Fisher's Exact Test. Continuous variables are presented as median values and ranges, and group comparisons were analyzed using the Mann-Whitney *U*-test. Methacholine and mannitol responses were log-transformed before calculations and are presented as geometric means.

For principal component analysis (PCA), data from spot urine samples 1 and 2 were averaged prior to log transformation and Pareto scaling using SIMCA-P+11 (Umetrics AB, Umeå, Sweden). Complete measurements of serum baseline tryptase and urinary mediators were available from 35 of the 39 subjects included in the study, and these 35 were therefore included in the PCA analysis.

Results

Subject characteristics

The characteristics of included subjects are shown in Table 1 and Table 2, including history of anaphylaxis. All SM patients were classified as indolent variant of disease (ISM). Further, patients were categorized according to the presence/absence of skin lesions, i.e. ISM with/without skin lesions (ISM s+/s-). Thirteen SM patients had skin engagement (ISM s+). In addition, eight patients in the study group experienced anaphylaxis, of whom six patients belonged to the ISM s+ group. Atopic sensitization was determined in 6 (43%) patients with SM, most of whom exhibited sensitization to more than one allergen. However, not all sensitized patients demonstrated clinical symptoms of allergic disease. We identified atopic disease in only three (21%) patients with SM (one had asthma, one rhinoconjunctivitis, and one both asthma and rhinoconjunctivitis).

Levels of tryptase, *te/e*-MIAA and 11β – PGF_{2α} were significantly higher in patients with SM than controls (Table 2 and Fig. 1A, B, C). The SM patients were identified as a distinct group in principal component analysis (Fig. 1D).

Skin reactivity

The median wheal size induced by skin prick test with the MC secretagogue morphine (10 mg/ml) was similar in all three groups (SM - 3.8 mm (range, 1-5 mm); healthy controls - 4 mm (range, 1-6.5 mm); asthma - 4 mm (range, 2.5-7 mm) (Fig. 2A); as was that induced by direct end-organ challenge with histamine (SM - 4 mm (range, 3-7 mm); healthy controls - 5 mm (range, 3-7 mm); asthma - 5 mm (range, 3.5-6.5 mm)) (Fig. 2B). When SM patients with or without anaphylaxis were analyzed separately, their MC reactivity was similar to that of controls (Fig. 2A, B). The skin prick test for all four patients with SM who exhibited venom-induced anaphylaxis was negative for bee and wasp venom, but gave a positive result by ImmunoCAP.

Pulmonary function and airway responsiveness

Baseline pulmonary function, expressed as FEV₁% predicted, did not differ between healthy controls and subjects with mastocytosis or asthma. Similarly, levels of exhaled nitric oxide (FeNO) did not differ between patients with mastocytosis compared to healthy controls (Table 2).

Similar to responses in the skin, we did not observe increased airway responsiveness towards mannitol or methacholine in the SM patients. Challenge with inhaled mannitol only produced a positive reaction (at a dose below the established cut-off value of ≤635 mg) in the single SM patient

also known to have asthma (Fig. 3A). For the methacholine challenge, two patients with SM (2/13) and no known asthma reacted to doses slightly below the cut-off value of 894 μ g (Fig. 3B).

Basophil histamine release assay

When basophil reactivity was assessed by measuring histamine release from peripheral whole-blood basophils in the presence of increasing concentrations of anti-IgE, the response in both healthy controls and SM patients was pronounced and dose-dependent with no difference between these groups (Fig. 4).

Discussion

Mastocytosis is characterized by a pathological release of mast cell mediators in various organs and tissues, thereby inducing mediator-related local or systemic clinical symptoms. However, it remains unclear whether the elevated MC mediator levels relate to an increased activation of tissue MCs, or increased numbers of MCs in various organs and tissues, or a combination of both. In this study, we confirm the presence of elevated MC mediators in the blood and urine of patients with SM (5, 6, 8). Nonetheless, the patients exhibited the same responsiveness to stimuli that cause local activation of mast cells in the skin and airways as that observed in healthy control subjects. Likewise, end-organ responsiveness, as caused by injection of histamine in skin or inhalation of methacholine in the airways, was the same as in control subjects or within normal ranges when compared to reference values.

We assessed both target-organ reactivity and local mast cell responsiveness in the skin. Histamine was injected to assess whether the skin directly exhibited altered responsiveness to this mast cell mediator. Upon injection, the mast cell secretagogue morphine also causes a wheal and flare response that is due to the release of histamine and other mast cell mediators from skin mast cells (20). However, skin responses to both mast cell activation and the direct effects of histamine were the same in controls and patients with SM. The current findings regarding the effects of morphine confirm and extend previous observations (10), supporting the conclusion that skin mast cells from SM patients do not have an altered threshold for triggering the release of mast cell mediators. The observation of unaltered histamine responsiveness argues against receptor tachyphylaxis or reduced histamine receptor expression as an adaptive mechanism in patients exposed to excessive mediator levels. Interestingly, all four SM patients with venom-induced anaphylaxis and the presence of venom-specific IgE in serum, exhibited negative skin prick tests towards the venom allergen, suggesting that the responsiveness of skin MCs to specific IgE-mediated stimuli may be attenuated.

As in the skin, airway reactivity was assessed in response to a directly acting bronchoconstrictor as well as indirectly following local mast cell activation. Although histamine is a well-known bronchoconstrictor, pulmonary function and airway responsiveness in patients with SM have not previously been investigated systematically. The standardized methacholine challenge test used in the current investigation causes direct activation of airway smooth muscle cells independent of mast cell activation, similar to histamine. In contrast, inhalation of the osmotically active substance mannitol activates airway mast cells and causes a bronchoconstriction that is due to the local release of mast cell mediators (21). However, we found no general increase in responsiveness towards methacholine among the patients with mastocytosis, nor responsiveness to mannitol. However one patient with

asthma did show increased responsiveness to mannitol. The two patients with mastocytosis that reacted to methacholine did so only at a high dose that is within the normal variability range for non-asthmatic subjects (18). Incidentally, our findings which do not show increased airway responsiveness in patients with mastocytosis are in line with the only previous assessment of airway function in these patients, where responsiveness to ultrasonically delivered distilled water, also a mast cell activating stimulus, was normal in a case study (22).

Although it is known that basophils share complementary and partially overlapping functions with mast cells, and also have the capacity to produce certain mast cell mediators including histamine, basophil reactivity has to the best of our knowledge not previously been investigated in patients with mastocytosis. Theoretically, it is possible that basophils may also be dysregulated and for this reason we investigated general basophil hyper-reactivity to test the hypothesis that basophils could be an additional source of excess histamine. We utilized the whole blood basophil histamine release test to provide an *in vitro* correlate of *in vivo* reactivity, and to explore whether basophils show hyper-reactivity in SM patients. However, we did not observe altered basophil reactivity towards IgE-receptor activation in patients with mastocytosis.

Our findings, which demonstrate that *in vivo* mast cell activity in SM patients is not increased in response to morphine or mannitol, suggest that the elevated numbers of tissue mast cells in these patients could explain the increased MC mediator levels. In addition, SM patients might have enhanced “leakage” of tryptase and histamine which are stored in granules. Such piecemeal release of mediators could occur in the absence of trigger-factor evoked activation of mast cells, a mechanism elucidated for tryptase (23), but not for histamine or other granule mediators.

Nevertheless, the clinical finding that patients with mastocytosis only experience symptoms periodically requires an explanation other than aberrant MC reactivity, or excessive release of mediators. One possibility is variable local or systemic production of protective substances such as PGE₂, which is bronchoprotective in the airways of asthmatic patients (24). This protective mechanism has recently been shown to involve mast cell stabilization (25).

There are limitations that may affect interpretation of the current study. Firstly, mastocytosis is a rare disease. Although cases were collected from a specialist referral centre over a three year period, only 15 new patients agreed to participate. However, the group was carefully diagnosed according to international criteria and displayed elevated levels of tryptase and other mast cell mediators, well-known disease characteristics. The seemingly contrasting, unaltered reactivity of skin and airways observed in the current study is therefore likely to be a valid general finding in mastocytosis, in line with the only previous study of skin reactivity in mastocytosis (10). Secondly, the study does not permit conclusions to be drawn regarding the level at which systemically elevated mast cell mediators have been generated. In addition to the hypothesis simply relating this to an increased number of total body mast cells, there might be a dysfunctional regulation of biosynthesis, storage or release of the mast cell mediators. This study was not designed to assess such possibilities. Thirdly, we are aware that SM patients are more prone to cardiovascular effects of mediator release rather than respiratory effects, and pathological mast cells are known to

accumulate around blood vessels. SM patients are also more prone to MC activation in response to physical stimuli such as friction, temperature changes and exercise rather than pharmacological stimuli. Opioid receptor-mediated mast cell activation (as examined in the skin) may not necessarily reflect changes in activation thresholds for responses towards physical stimuli, or the responsiveness of mast cells at sites other than the skin.

In summary, our study reveals that MCs in the skin and airways of subjects with SM are not hyper-reactive to the MC-activating stimuli morphine and mannitol respectively, and nor are these target organs hyper-responsive as assessed by challenge with histamine and methacholine respectively. Finally, no evidence of increased basophil reactivity was detected in these patients. We conclude that the remarkably elevated baseline levels of MC mediators in patients with SM are most likely related to the large numbers of MCs present, and probably by leakage of mediators from these. However, other dysfunctions in mediator synthesis, storage and release, or MC reactivity towards other types of stimuli cannot be ruled out. Furthermore, the clinical finding that patients with mastocytosis only experience symptoms episodically warrants further investigation.

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Conflict of interest statement

None of the authors have relevant conflicts of interest to declare.

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Figure legends

Fig. 1. A: Baseline serum tryptase levels (sBT). B: Urinary levels of the major histamine metabolite tele-MIAA. C: Urinary levels of 11β -PGF $_2\alpha$, a metabolite of PGD $_2$ in healthy controls and subjects with asthma or systemic mastocytosis (SM). The values presented are medians with interquartile ranges. D: Principal component analysis (PCA) of the relationships between tryptase, tele-MIAA and 11β -PGF $_2\alpha$ based on variance analysis. These variables were log-transformed and Pareto scaled. The extent of the variance explained by PC1 and PC2 was 79 and 14%, respectively. The extensive increase in their concentrations in some of the SM patients is reflected in the spread along PC1 axis. The SM patients formed a cluster that was clearly distinguishable from both the patients with asthma and the healthy controls.

Fig 2. A: Skin reactivity to morphine and **B:** histamine as assessed by the skin prick test in healthy controls and subjects with asthma and systemic mastocytosis (SM). In addition, SM patients with or without anaphylaxis (AX) were analyzed separately. The values presented are medians with interquartile ranges.

Fig. 3. A: Airway reactivity to inhalation challenge with mannitol or **B:** methacholine in 15 subjects with systemic mastocytosis (SM) and 4 subjects with asthma. The values presented are geometric means with 95% CI and the dashed lines indicate the cut-off levels (PD₁₅ positive <635 mg for mannitol and PD₂₀ positive <894 µg for methacholine). To illustrate airway hyper-responsiveness to direct and indirect challenges we have included four subjects with asthma in the figure.

Fig. 4. Histamine release by blood basophils from healthy control subjects and patients with systemic mastocytosis in the presence of 0.01 - 5 µg/ml anti-human IgE (α-hIgE). The values are presented as boxes with whiskers (minimum to maximum).

Table 1: Characteristics of patients with systemic mastocytosis

Patient ID, Gender	Age	Skin lesions	Hx of anaphylaxis	Elicitor	MC clusters	Atypical MCs	CD2/25	D816V KIT mutation	sBT (µg/L)	IgE (kE/L)	Atopy
1, F	46	ISM s+	No	n/a	No	Yes	-/+	Positive	26	3.6	No
2, F	43	ISM s+	Yes	Unknown	Yes	Yes	-/+	Positive	7.6	16	Yes
3, M	53	ISM s+	Yes	Unknown	No	Yes	+/+	Positive	25	13	No
4, M	64	ISM s+	Yes	Wasp SPT- /CAP+	Yes	Yes	-/+	ND	25	16	No
5, F	68	ISM s+	Yes	Wasp SPT- /CAP+	No	No	+/+	Positive	15	89	Yes
6, F	21	ISM s+	No	n/a	No	Yes	+/+	Positive	12	19	No
7, F	63	ISM s+	Yes	Food (shrimp) SPT- /CAP+	Yes	Yes	+/+	Positive	36	25	Yes
8, F	68	ISM s+	No	n/a	Yes	Yes	+/+	Positive	81	3,4	No
9, F	69	ISM s+	No	n/a	Yes	Yes	-/+	Positive	320	3,2	No

10, M	43	ISM s-	Yes	Wasp SPT- /CAP+	No	Yes	+/+	Positive	13	140	Yes
11, F	42	ISM s+	No	n/a	Yes	Yes	+/+	Positive	217	1	No
12, F	48	ISM s+	No	n/a	Yes	Yes	-/+	Positive	120	5,7	No
13, F	32	ISM s+	No	n/a	No	Yes	-/+	Positive	18	8,6	No
14, M	45	ISM s-	Yes	Unknow n	Yes	Yes	+/+	Positive	160	18	Yes
15, M	65	ISM s+	Yes	Wasp SPT- /CAP+	No	No	+/+	Positive	27	44	Yes

ISM, indolent systemic mastocytosis; ISM s+/-, ISM patients with/without skin engagement; MC, mast cell; Hx, history; n/a, not applicable; ND, not done; SPT, skin prick test; sBT, serum baseline tryptase

Table 2: Demographic data and characteristics of the study subjects

Characteristics	SM (n=15)	Asthma (n=11)	Healthy (n=13)
Age, yr <i>median (range)</i>	48 (21-69)	30 (22-52)	33 (22-55)
Gender, M/F (M %)	5/10 (33 %)	8/3 (73 %)	5/7 (42 %)
Presence of atopy, <i>n (%)</i>	6 (43 %) 1 ND	11 (100 %)	0
Atopic disease, <i>n (%)</i>	3(21 %)	11 (100 %)	0
Occurrence of anaphylaxis, <i>n (%)</i>	8 (53 %)	N/a	N/a
Levels of total IgE kE/l, <i>median (range)</i>	16 (1-140)	85 (21-2500)	17 (6.2-36)
Levels of sBT ng/ml, <i>median (range)</i>	26 (7.6-320)	4.5 (2.1-13)	5.3 (1-15)
Levels of <i>tele</i> -MIAA μmol/mmol-crea <i>median (range)</i>	5.4 (2.65-30.15)	2.75 (1.8-4.8)	2.75 (1.8-3.7)
Levels of 11β-PGF _{2α} ng/mmol-crea <i>median (range)</i>	109 (44-468)	64 (25-151)	48 (32-128)
FEV ₁ (% of expected) <i>mean (range)</i>	103 (77.4-128)	98 (72.6-121)	109 (88-133.9)
Levels of FeNO ppb <i>median (range)</i>	15 (4-37)	28 (17-72.7)	14 (8-69)

SM, systemic mastocytosis; ND, not determined; sBT, serum baseline tryptase; MIAA, 1-methyl-4-imidazoleacetic acid; FEV₁, forced expiratory volume in one second; FeNO, fraction of exhaled nitric oxide; ppb, part per billion.

















