

ORIGINAL ARTICLE

EXPERIMENTAL ALLERGY AND IMMUNOLOGY

Urinary 11 β -PGF 2α and N-methyl histamine correlate with bone marrow biopsy findings in mast cell disorders

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To cite this article: Divekar R, Butterfield J. Urinary 11 β -PGF 2α and N-methyl histamine correlate with bone marrow biopsy findings in mast cell disorders. *Allergy* 2015; DOI: 10.1111/all.12668

Keywords

11 β -prostaglandin F 2α ; *c-kit* mutation; Mastocytosis; N-methyl histamine; Tryptase.

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Accepted for publication 5 June 2015

DOI:10.1111/all.12668

Edited by: Marek Sanak

Abstract

Background: The utility of measuring histamine and prostaglandin metabolites in the urine of patients with mastocytosis has not been critically examined in a large series of patients. This study examined the relationship between the extent of increase in urinary excretion of 11 β -prostaglandinF 2α and N-methyl histamine, with serum tryptase, whole blood serotonin, and bone marrow findings including morphology, percentage involvement, and abnormal mast cell phenotype.

Methods: This was a retrospective analysis of 90 patients who were continuously enrolled in the study for a period of 6 years (2008–2014). We recorded serum tryptase, whole blood serotonin, levels of urinary mast cell metabolites 11 β -prostaglandinF 2α and N-methyl histamine (NMH), and bone marrow findings.

Results: Urinary mast cell metabolites 11 β -prostaglandinF 2α and N-methyl histamine correlated with levels of serum tryptase, mast cell burden in the bone marrow, the presence of mast cell aggregates, and atypical mast cells on bone marrow biopsy. Whole blood serotonin did not have a significant correlation with the serum tryptase or mast cell burden in the bone marrow. Urinary NMH was significantly different between *c-kit* D816V-positive and *c-kit* D816V-negative patients, while 11 β -prostaglandinF 2α was not. Urinary 11 β -prostaglandinF 2α 24-h excretion >3500 ng and NMH levels >400 μ g/gm Cr corresponded with the high degree of bone marrow biopsies positive for atypical mast cells, the presence of aggregates, and *c-kit* mutation.

Conclusions: Easily obtained and quantified urinary metabolites of histamine (greater than twice the upper limit of normal) and prostaglandin D $_2$ (>3.4 times the upper limit of normal) correlate well with bone marrow findings of mastocytosis.

Formal criteria to define systemic mastocytosis (SM), including the major criterion (multifocal dense infiltrates of >15 mast cells/infiltrate in a tryptase-stained bone marrow biopsy) and minor criteria (>25% of mast cells showing a spindle or other abnormal morphology; abnormal phenotype of bone marrow mast cells as defined by the presence of CD2

and/or CD25; the presence of the *c-kit* mutation Asp816Val (D816V); serum tryptase value >20 ng/ml), have relied on a narrow range of test results(1, 2). As currently defined, the presence of the major criterion plus one minor criterion or the presence of 3 minor criteria constitutes a diagnosis of SM (3). However, majority of the criteria except for serum tryptase rely on bone marrow biopsy which is invasive. Noninvasive biomarkers to assess for mast cell disease have been studied but have not been brought into mainstream diagnostic algorithms.

Serum tryptase levels have been used to estimate the burden of mast cells (MC) in SM and to help distinguish between categories of disease with the highest tryptase levels found in MC leukemia, followed by smoldering SM, aggressive SM, and indolent SM (4); however, there is substantial

Abbreviations

11 β -PGF 2α , 11 β -prostaglandin F2 alpha; ASM, aggressive systemic mastocytosis; CM, cutaneous mastocytosis; IQR, interquartile range; ISM, indolent systemic mastocytosis; LTE4, leukotriene E4; MCAS, mast cell activation syndrome; NMH, N-methyl histamine; SM-AHNMD, systemic mastocytosis with associated clonal hematologic non-mast cell lineage disorder; SSM, smoldering systemic mastocytosis.

overlap between disease categories. Tryptase levels have also been used as markers for increased risk of SM in patients with systemic reactions to hymenoptera stings (5), as an indication of mast cell activation in anaphylaxis and mastocytosis (6) and as one of the criteria utilized to define mast cell activation syndrome (7). Serum tryptase levels have been used to monitor the response to mast cell cytoreductive therapy (8); however, other studies have called into question the validity of following tryptase values for this purpose (9). Because the serum tryptase level can be elevated in myeloid neoplasms (10), it cannot be used as a minor criterion in patients having SM with an associated clonal hematologic nonmast cell lineage disease which commonly is a myeloid neoplasm (SM-AHNMD) (11). Hence, in this subset of patients, additional tests could be useful to support a diagnosis of SM.

Other markers besides tryptase such as histamine and prostaglandin D₂ overproduction in SM have been reported for over 30 years, but the correlation of these levels with current criteria for SM is not utilized. In patients with SM, plasma histamine values exhibit a diurnal variation with the highest level present in the early morning. Although patients with SM on average have elevated plasma histamine levels, determination of plasma histamine alone has not proven useful to screen patients for SM (12). Measurement of urinary histamine metabolites N-methyl histamine (NMH) and N-methylimidazoleacetic acid rather than measurement of urinary histamine has proven more useful in supporting a diagnosis of SM (13, 14). Interestingly, similar to the findings of elevated tryptase in myeloid neoplasms referred to above, persistent elevated 24-h excretion of histamine metabolites has also been found in chronic myelocytic leukemia and polycythemia vera (14). One study found no relationship between histamine metabolite excretion and serum levels of tryptase in SM (15). Neither serum histamine nor an increased level of urinary histamine metabolites is currently a criterion for diagnosing SM.

Mast cells are the primary source of prostaglandin (PG) D₂ although there are other cellular sources of PGD₂ in humans (16–21). Increased PGD₂ production has been linked to the flush syndrome in systemic mastocytosis (22). Increased production of PGD₂ and detection of its major metabolite in SM have been verified in several reports (23–26). Increased quantities of PGD₂ are released in the skin following the administration of nicotinic acid (27, 28), an effect that can be blocked by aspirin administration (29). At present, neither the serum PGD₂ level nor urinary level of PGD₂ metabolites is included among criteria for defining SM.

Both serotonin and its 5-HT_{1A} receptor are expressed on mast cells from biopsies of mastocytomas, skin involved in urticaria pigmentosa, and on HMC-1 cells (30). Serotonin levels have been examined in SM. In one study, total blood serotonin levels followed a bimodal distribution in SM, unlike a normal distribution pattern found in normal volunteers. Lower serotonin levels were seen in individuals with higher rates of fatigue, migraine headaches, psychiatric symptoms, diarrhea, flushing, and abdominal and bone pain, but

did not correlate with serum tryptase levels, liver function tests, or platelet numbers (31). We (JB) reported in a small study that normal whole blood serotonin (WBS) levels were detected in cutaneous mastocytosis (CM) (six patients), SM (25 patients), and mast cell activation syndrome (MCAS) (five patients), and no association was found between WBS levels and any other mast cell mediator level or with platelet counts (32). Finally, sustained paranoid psychosis has been reported in one patient with mast cell leukemia and a decreased serotonin level (33). As most WBS levels in SM are normal or low, their utility in diagnosing SM has remained problematic.

Despite the potential importance of detecting these mediators in evaluating patients suspected of having SM and in determining which mast cell product(s) are responsible for symptoms, there are surprisingly few reports that examine whether levels of these mediators could be predictive of SM. Here, we show the side-by-side correlation of tryptase, whole blood serotonin, and urinary NMH and 11 β -PGF_{2 α} and select NMH and PGD₂ metabolites (11 β -PGF_{2 α}) as noninvasive biomarkers to study their relation to specific bone marrow findings in mast cell disease.

Material and methods

Study design

This was a retrospective study of participants enrolled in a review of whole blood serotonin levels in mastocytosis and other disorders of potential mast cell activation. Of all the participants, those who had abnormal serum tryptase (>11.5 ng/ml) at the time of measurement of whole blood serotonin, and urinary NMH and 11 β -PGF_{2 α} were included in the analysis. The study was approved by the institutional review board of the Mayo Clinic.

Measurement of serum tryptase, serotonin, and urinary NMH and 11 β -PGF_{2 α}

Values of serum tryptase, whole blood serotonin, and urinary metabolites collected were measured at Mayo Medical laboratories. Tryptase was measured by fluorescence enzyme immunoassay. WBS was measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Urinary 11 β -prostaglandin (PG)F_{2 α} was measured by direct immunoassay (EIA, Commercial Kit, Cayman chemical company, Ann Arbor, MI, USA) and urinary NMH by LC-MS/MS on 24-h urine collections (34).

Biopsy results

Bone marrow biopsies were obtained in the course of evaluation for SM or MCAS. Biopsies were stained for mast cell quantitation with tryptase and examined for the presence of CD2 and CD25 by immunohistochemistry. Bone marrow was examined for the presence of the *c-kit* D816V mutation by allele-specific oligonucleotide polymerase chain reaction (PCR).

Statistical analysis

Wilcoxon rank-sum test was performed for the pairwise comparison of continuous data, while Fisher's exact test was used for the comparison of ordinal data. For the correlation analysis of continuous data, Spearman's coefficient was calculated. For line of fit modeling, R value and significance were computed. Two sided P values <0.05 were considered statistically significant. Statistical analysis and graphing were carried out using JMP software (v10, SAS institute Inc, Cary, NC, USA) and Microsoft Office (2010 Microsoft corporation, Redmond WA, USA).

Results

Characteristics of the study population

Table 1 elucidates the characteristics of the patients in this study. About 90 patients were included in the study, and all had been referred to the Mayo Clinic Program for mast cell and eosinophil disorders. Mean age of patients was 48 years, and male to female ratio was approximately proportional. Tryptase levels ranged from 11.8 to 517 ng/ml. Biopsy reports were available for 78 patients, tryptase on 90 patients, and urinary metabolites on 89 patients. There were no statistical differences between male and female subjects with respect to age, urinary NMH concentration, 11 β -PGF2 α 24-h excretion, serum tryptase levels, or the biopsy parameters studied ($P > 0.1$). Final distribution of mast cell diseases after full work-up was as follows: smoldering SM (SSM) (1), smoldering SM with an associated hematologic nonmast cell lineage disease (SSM-AHNMD) (1), 'not classified' (NC) (9), mast cell activation syndrome (MCAS) (7), indolent SM (ISM) (63), cutaneous mastocytosis (CM) (7), ASM-AHNMD (1), and aggressive SM (ASM) (1). The NC category included patients in whom mast cell mediator release symptoms were present and tryptase was elevated > 11.5 ng/ml but did not meet the formal criteria of mast cell disease or MCAS. These included final working diagnosis of osteoporosis (1), cyclic vomiting with lymphocytic colitis (1), granuloma annulare (1), aleukemic mast cell leukemia (1), food intolerance/allergy (1), myeloid neoplasm (1), and 'spells' (3) (clinical features suggestive of mediator release). For patients with ISM, median tryptase was 42.4 (IQR 23.7–70.8), median age was 46 (IQR 39–56.5), and gender compositions with male and female were 45% and 54%, respectively, while for patients in the non-ISM grouping, median tryptase was 24.1 (IQR 16.1–45.6), median age was 53 (IQR 48–61), and similar gender compositions with male and female were 52% and 47%, respectively.

Urinary mast cell mediator metabolites 11 β -PGF2 α and NMH correlate with serum tryptase

To evaluate the selected clinical markers of mast cell activity (11 β -PGF2 α , NMH, and whole blood serotonin), their relationship with serum tryptase was analyzed. Total 11 β -PGF2 α (ng/24 h) (Fig 1A), NMH (Fig. 1B), and whole blood

Table 1 Characteristics of the patients in current study

Parameter	Value
Total number	90 patients
Age (at biopsy) years	Range 10–81, mean 48.8 ± 1.6
Gender*	42 females, 38 males
Tryptase (ng/ml)	11.8–517, mean 62.8 ± 8.8 SEM
N-methyl histamine (μ g/gm Cr.)	46–9772, mean 779.4 ± 131.9 SEM
11 β -PGF2 α (ng)/24 h total	154–16 641, mean 2466.5 ± 289 SEM
c-kit D816V reported	Total $n = 73$, negative = 19, positive = 54
Mast cell morphology	Total $n = 78$, atypical = 64, not atypical = 14
Mast cell collections	Total 77, aggregates = 52, Aggregates not reported = 25

*There was no statistical difference between the genders with respect to age, NMH, PGF2 α levels, total 24-h 11 β -PGF2 α concentration, tryptase level, or the biopsy parameters studied.

serotonin (Fig 1C) were compared to serum tryptase in subjects. We see a positive correlation between serum tryptase and urinary 11 β -PGF2 α total 24-h excretion (overall correlation ρ , 0.33, $P = 0.0015$, while $R = 0.311$, $P = 0.003$ for line of fit) and NMH ($\rho = 0.71$, $P < 0.0001$ and $R = 0.51$, $P < 0.001$ for line of fit). WBS levels did not correlate with serum tryptase ($\rho = -0.17$, $P = 0.11$) and additionally, there was no relationship between WBS and NMH ($\rho = -0.12$, $P = 0.24$) or 11 β -PGF2 α ($\rho = 0.02$, $P = 0.81$) (data not shown). The patient with myeloid neoplasm mentioned above under 'characteristics of the study population' warrants comment. This patient (70% mast cells in marrow, c-kit D816V negative, CHIC2/PDGFR α positive, JAK V617F negative, CD25+, CD2– phenotype, atypical mast cell in biopsy exam with CD117+ and tryptase + aggregates seen) had excretions of NMH and 11 β PGF2 α that exceeded, respectively, seven and eight times the upper limit of normal (NMH: 1462 mcg/g creatinine, 11 β -PGF2 α : 8024 ng/24 h). The NMH elevation is not wholly unexpected as elevations in histamine metabolites have previously been reported in chronic myelomonocytic leukemia and polycythemia vera (14). Therefore, in the presence of a myeloid neoplasm, NMH elevations may need to be interpreted with caution when used to screen for systemic mastocytosis or MCAS. Elevated levels of PGD2 metabolites have not been reported in myeloid neoplasms, and mast cells, but not basophils, remain a major source for PGD2 (35). Therefore, elevated levels of 11 β PGF2 α may be a more reliable test for mast cell proliferation in certain clinical situations.

Urinary mast cell mediator metabolites 11 β -PGF2 α and NMH correlate with mast cell burden in the bone marrow

As the measurement of urinary 11 β -PGF2 α and NMH had a direct correlation with the level of serum tryptase, we assessed the relationship between these mediators to the mast cell burden in the bone marrow (Fig 2). Mast cell burden was

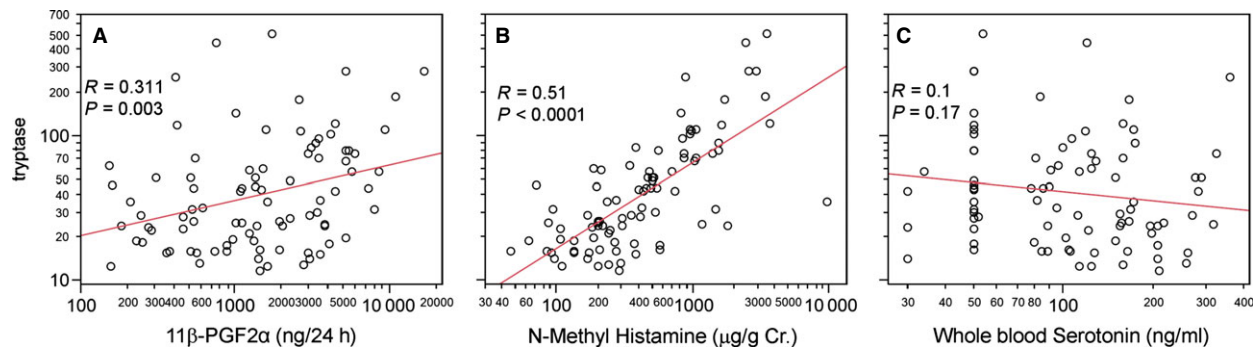


Figure 1 Urinary mast cell mediator metabolites 11 β -PGF2 α and N-methyl histamine correlate with serum tryptase (ng/ml). (A), Urinary levels of 11 β -PGF2 α (ng per 24 h collection) plotted against serum tryptase (ng/ml). (B), Concentration of N-methyl histamine

(μ g/gm Cr.) in urine and correlation with serum tryptase levels. (C), Whole blood serotonin and correlation with serum tryptase. Line of fit is log-log to account for logarithmic scales. *P* values are represented along with *R* coefficient.

measured as the percentage (%) of mast cells reported on biopsy. There is a direct and significant correlation between the percentage of mast cells on bone marrow biopsy and levels of urinary total 11 β -PGF2 α excretion /24 h (overall correlation ρ , 0.43, *P* = 0.0006 and for line of fit *R* = 0.39, *P* = 0.002) and urinary NMH levels (ρ = 0.69, *P* < 0.0001 and for line of fit *R* = 0.56, *P* = 0.001). Whole blood serotonin levels again did not correlate with mast cell burden (ρ = -0.07, *P* = 0.59) in the bone marrow at the time of biopsy. As a control, tryptase

levels had a high correlation with mast cell burden (ρ = 0.45, *P* = 0.0004 and for line of fit, *R* = 0.44, *P* = 0.0004, Fig. 2B).

Urinary mast cell mediator metabolite 11 β -PGF2 α correlate with mast cell atypia and aggregates but not with the presence of *c-kit* D816V mutation

As there was a strong correlation between the mast cell burden in the bone marrow and levels of 11 β -PGF2 α and

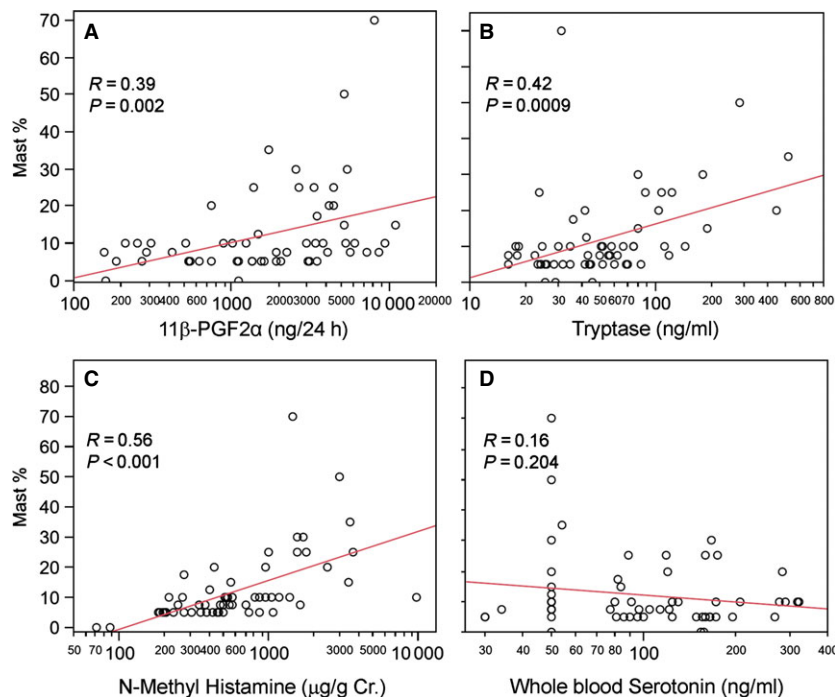


Figure 2 Urinary mast cell mediator metabolites 11 β -PGF2 α and N-methyl histamine correlate with mast cell burden in the bone marrow. Mast % is the percentage of mast cells in the bone marrow biopsy (Y axis, linear), while 11 β -PGF2 α levels, total 24 h

excretion (A), serum tryptase (B), N-methyl histamine (C), and whole blood serotonin (D) are the percentages of mast cells in the bone marrow biopsy on X-axis (Log). Line of fit is lin-log. *P* values are represented along with *R* coefficient.

NMH in urine, we further analyzed the relationship between these metabolites and mast cell morphology. Total 24-h excretion of 11β -PGF 2α was different in patients with mast cell atypia or aggregates on bone marrow biopsy. Patients with 'atypical' mast cells had higher 11β -PGF 2α levels (median 1932.5 ng/24 h [562–3984.75] IQR) compared to those where atypia was not seen ('not atypical') (median 978 ng/24 h [413.5–1395] IQR, $P = 0.01$). There was also a significant difference between those patients reported to have compact infiltrates of mast cell (median 1669 ng/24 h [632.75–4109.25] IQR) vs those with loosely distributed cells (median 1045 ng/24 h [460–2204.25] IQR, $P = 0.04$).

For urinary NMH (Fig 3, lower panels), there was a significant difference between subjects reported to have atypical mast cells (median 499 μ g/g Cr [281–1059 IQR]) vs biopsies that did not report as such (median 106 μ g/g Cr [79.5–134 IQR], $P < 0.0001$). NMH levels were higher in patients with compact infiltrates of mast cells (median 530 μ g/g Cr [281–1146 IQR]) compared to those with loosely distributed mast cells (median 203.5 μ g/g Cr [99.25–384.25 IQR], $P < 0.0001$).

Correlation between urinary 11β -PGF 2α and NMH levels and the presence of the *c-kit* D816V mutation was examined in seventy-three patients who had *c-kit* mutations analyzed in the bone marrow (*c-kit* negative $n = 19$, *c-kit* positive $n = 54$ [Table 1]). As shown in Fig. 3 (right column), total 24-h excretion of 11β -PGF 2α was not statistically different between *c-kit* mutation-positive and *c-kit* mutation-negative patients. NMH levels, however, were statistically different between the group of patients that were positive for *c-kit* mutation (median 451 μ g/g Cr [247.5–907.5 IQR] vs the

group that did not have the mutation (median 186 μ g/g Cr [106–537 IQR], $P = 0.01$).

Discriminant function of increasing urinary mast cell mediator metabolites and relationship with bone marrow findings

To assess whether increasing concentrations of urinary mast cell metabolites discriminate biopsy findings, 11β -PGF 2α and NMH levels were divided into quartiles (Q1 to Q4-left to right, Fig. 4A–C) and proportions of subjects with mast cell atypia, mast cell aggregates, and *c-kit*-positive mutations in the bone marrow studied.

NMH

For patients with NMH values <200 μ g/g Cr ($n = 22$) (Q1), mast cell atypia (33%), aggregates (38%), and *c-kit* positivity (44%) were seen in a low proportion of patients. For values between 201 to 400 μ g/g Cr ($n = 24$) (Q2), there was a marked increase in the proportion of atypia (94%), mast cell aggregates (52%), and *c-kit* positivity (83%). This increase was significant compared to Q1 for atypia ($P = 0.0001$) and *c-kit* positivity ($P = 0.03$) but not for the presence of mast cell aggregates compared to Q1 patients. Further increase in urinary NMH 401 to 886 μ g/g Cr. ($n = 22$) (Q3) corresponded with atypia (95%), the presence of aggregates (85%), and *c-kit* positivity (90%). Increase in the proportion of biopsies with aggregates between Q2 and Q3 was significant with $P = 0.04$ (Fig. 4A, left middle panel). For subjects with NMH levels >949 μ g/g Cr. ($n = 22$) (Q4), atypia was seen in 100% biopsies, aggregates in 90%, and *c-kit* mutation in 76.4%.

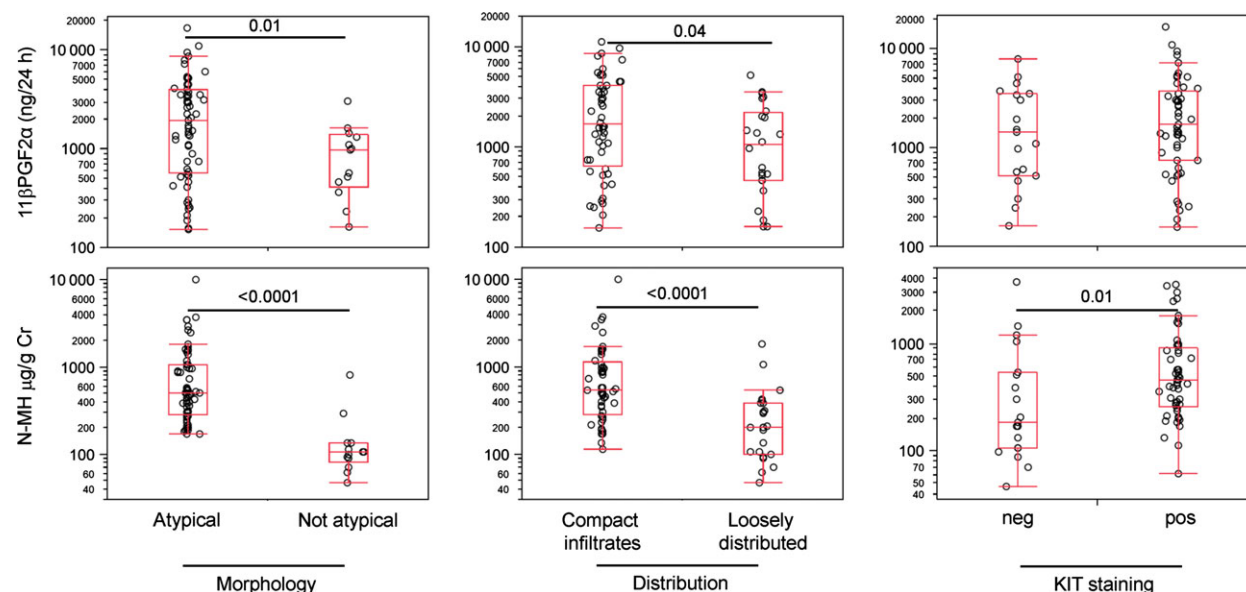


Figure 3 Urinary mast cell mediator metabolites 11β -PGF 2α and N-methyl histamine correlate with abnormal mast cell pathology. Increased urinary N-methyl histamine but not 11β -PGF 2α excretion correlated with the presence of *c-kit* D816V mutation. Levels of

11β PGF 2α total 24-h excretion (upper) and N-methyl histamine (lower panels) were analyzed in relation to mast cell morphology (left), distribution (middle), or the presence of *c-kit* mutation (right column) in biopsy.

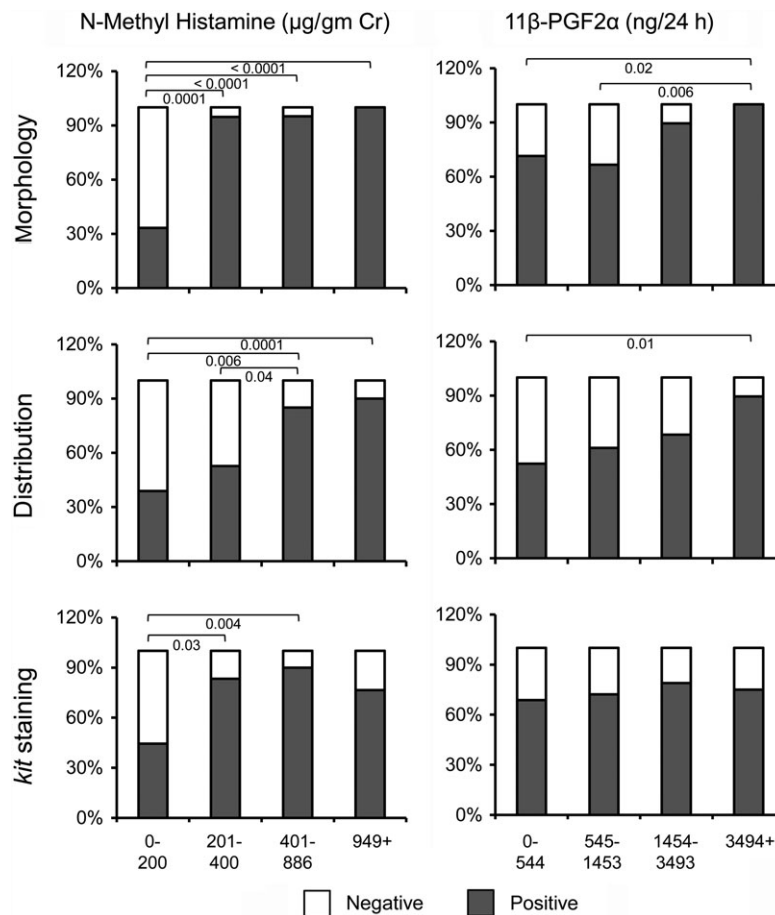


Figure 4 Discriminant function of increasing urinary mast cell mediator metabolites and relationship with bone marrow findings. Levels of N-methyl histamine and total 11β-PGF2α excretion were divided into quartiles (Q1–Q4). Findings of atypical mast cells

(atypia), compact distribution (aggregates), and c-kit mutation (c-kit D816V) based on those interquartile ranges are depicted. Fischer's exact test used to calculate the significance in difference between groups. *P* values < 0.05 considered as significant.

11β-PGF2α

When the total 24-h excretion levels of 11β-PGF2α were >3494 ng/24 h (*n* = 22), atypical mast cells (100%), aggregates (89%), and *c-kit* positivity (75%) were all markedly increased.

Discussion

As currently defined, the WHO criteria to diagnose SM include tryptase elevation above 20 ng/ml, approximately twice the upper limit of normal (1, 2, 36); however, none of the other noninvasive biomarkers of mast cell function whose levels or activities have been reported to be increased in this disorder are currently included among the diagnostic criteria. These mediators include PGD2 (23–25), histamine metabolites (14, 37), heparin (38, 39), thromboxane B2 (37), IL-6 (40), and others. Perfect correlation of these mediators' levels with each other has not been forthcoming. This is not unexpected because many of these mediators can be produced by several cell types in addition to mast cells. For example, both

basophils (41) and mast cells synthesize histamine (42), and in addition to mast cells, there are other cellular sources of PGD2 in humans (16–21). A recent report has called for a re-assessment of the WHO criteria for SM, including the addition of other objective clinical and laboratory data. In this report, it was shown that of the current WHO criteria for SM, the serum tryptase level appears to be less sensitive than the others, with levels >20 ng/ml in only about 70% of cases (43). In another series, multifocal dense infiltrates of mast cells, the major criterion, were present in only 36 of 59 cases (68%) (44). Therefore, additional tests may be of use in diagnosing or predicting SM.

Although measuring whole blood serotonin may seem appealing in mast cell disorders as several symptoms seem common to SM and carcinoid syndrome including flushing, diarrhea, and nonspecific gastrointestinal symptoms such as bloating, we found no relationship between serum tryptase and WBS. Additionally, there was no relationship between WBS and other mediators like NMH or 11β-PGF2α or correlation with mast cell burden in the bone marrow at the

time of biopsy. This result supports prior studies which looked at WBS levels in SM (31, 32). This lack of correlation with whole blood serotonin is also in agreement with the presence of little serotonin in human mast cells compared to murine mast cells (45).

In this study, we show that the measurement of histamine and PGD2 metabolites correlate well with other SM criteria and together, high levels of these mediators can provide additional support for a diagnosis (or predict bone marrow findings) of SM. We found strong correlations between serum tryptase values and excretion of 11 β -PGF2 α and NMH.

The use of several mediators to diagnose SM is not without precedent. The combination of serum tryptase and urinary excretion of histamine metabolites has been utilized as a diagnostic indicator for patients who are suspect for ISM but who lack findings of mastocytosis in the skin (MIS) (46). In a similar fashion, serum tryptase values, combined with typical clinical symptoms or the c-kit D816V mutation (in peripheral blood), are featured in another diagnostic algorithm for screening patients who lack MIS but are suspected of having SM (47). In this latter report, tryptase values in the range of 15–30 ng/ml were considered mildly elevated although these levels overlap the minor criterion value of 20 ng/ml, which is about twice the upper limit of normal. In that report, only patients with tryptase values of >30 ng/ml suspected of having SM were considered for complete staging including bone marrow studies.

To date, there have been only rare attempts to simultaneously examine, in a large series, the relationships between urinary excretion levels of prostaglandin D2 metabolites, N-methyl histamine and the current biopsy criteria for SM despite the fact that these tests are both noninvasive and can be obtained from major clinical laboratories. Here, we show that urinary levels of both 11 β -PGF2 α and NMH correlate not only with serum tryptase, a minor criterion for diagnosing SM, but also with mast cell burden in the bone marrow, the presence of atypical mast cells, another minor criterion, and the presence mast cell aggregates, the major criterion for diagnosing SM. Furthermore, urinary NMH, but not 11 β -PGF2 α , correlated with the presence of the c-kit D816V mutation.

In discriminant analysis, excretion of NMH at levels approximately >4.7 times the upper limit of normal (949 μ g/gm Cr.) was associated with abnormal bone marrow findings of atypical cells (100%), aggregates (90%), and c-kit positivity (76.4%). For 11 β -PGF2 α levels >3.4 times the upper limit of normal (3494 ng/24 h), comparable

percentages were found for atypical mast cells (100%), aggregates (89%), and c-kit positivity (75%). For practicality and clinical applicability, NMH greater than twice the upper limit of normal (400 μ g/gm Cr, corresponding with 95% mast cell atypia and 85% mast cell aggregates finding) or 11 β -PGF2 α >3.4 times the upper limit of normal (3500 ng/24 h, corresponding with 100% atypia and 89% aggregates) may be clinically useful to predict bone marrow findings in mastocytosis.

There are some limitations to this study. Even though the patients were enrolled prospectively, the analysis of the mast cell clinical mediators and bone marrow findings was a retrospective chart review. It would have been ideal to obtain mediator levels exactly at the time of the bone marrow biopsy, but this has limited practical value as bone marrow biopsy of patients with mast cell disease may not always correlate with timing of other laboratory work. Furthermore, we could not control for the number of patients in each subgroup analysis as this was a retrospective study leading to disproportionate number of patients in some comparisons (c-kit). The strengths of the study include a comprehensive analysis of the serum tryptase levels, urinary 11 β -PGF2 α , urinary NMH, and whole blood serotonin in relation to biopsy pathology in mast cell disorders. Secondly, this study analyzes 90 subjects that were continuously enrolled in the study over a period of 6 years (2008–2014) allowing for the discrimination of differences between the markers studied. Prospective studies designed to calculate the predictive capacity of these noninvasive biomarkers would greatly enhance the diagnostic opportunities for this disease.

Acknowledgment

We wish to thank the participants enrolled in the study.

Funding

Mayo Clinic internal funds.

Author contributions

RD and JB were involved in the acquisition of data, analysis and interpretation of data, and drafting this manuscript.

Conflict of interest

The authors report no conflict of interest with this activity.

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