Clinical Commentary Review

# The Utility of Measuring Urinary Metabolites of Mast Cell Mediators in Systemic Mastocytosis and Mast Cell Activation Syndrome

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**Mast cells (MCs) leave evidence of their presence and activation. Aside from increased numbers of MCs in tissues, this evidence includes detecting elevated serum levels of tryptase and discovering increased excretion of urinary metabolites of prostaglandin (PG) $D_2$, leukotriene (LT) $C_4$, and/or histamine. The importance of measuring these nontryptase mediator metabolites has largely gone unnoticed. We reviewed the utility of quantitating urinary levels of MC mediator metabolites in systemic mastocytosis (SM) and MC activation disorders and summarized the relative production of these mediators by MCs and other cell types. Quantitation of urinary n-methyl histamine, 2,3-dinor-11βPGF$_{2\alpha}$, and $LTE_4$ offers a simple, noninvasive avenue to monitor their constitutive release as well as contemporaneous discharge during MC activation as well as supporting a diagnosis of SM. These increases can occur independently of or in addition to raised levels of tryptase. Quantitation of these mediator metabolites potentially offers targets for therapeutic intervention. Synthesis of $PGD_2$, a product nearly exclusively of MCs, can be controlled with aspirin, histamine increase by H1 and H2 receptor blockade, and $LTC_4$ by a 5-LO inhibitor or LT receptor antagonist. Although other sources are reported, the increase in MC numbers in SM supports this cell as the predominant origin of all 3 mediators.** © 2020 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2020;■:■-■)

***Key words:*** *Systemic mastocytosis; Mast cell activation syndrome; Histamine; N-Methyl histamine; Leukotriene $C_4$; Leukotriene $E_4$; Prostaglandin $D_2$; 2,3-Dinor-11β-PGF$_{2\alpha}$*

Systemic mastocytosis (SM), a clonal disorder of tissue infiltration by excessive mast cell (MC) numbers, and mast cell activation syndrome (MCAS), a disorder of episodic, or, more rarely, chronic symptoms due to release of an overabundance of MC mediators, continue to challenge the diagnostic and therapeutic expertise of clinicians.

The criteria for the definition of SM have resulted from the collaborative work of experts in the fields of allergic disorders, hematology, gastroenterology, dermatology, and pathology. As currently specified, the major criterion, bone marrow infiltration by compact clusters of 15 or more MCs in tryptase-stained specimens, and one of the minor criteria: (1) serum baseline tryptase level of ≥20 ng/mL; (2) the presence of the Asp816Val mutation in the MC *KIT* receptor; (3) abnormal MC morphology, a fibroblastic or spindled appearance, in >25% of bone marrow MCs; and (4) abnormal MC phenotype as indicated by the presence of CD25 on bone marrow MC, are required for the diagnosis of SM. Alternatively, in the absence of the major criterion, SM can be diagnosed by the presence of 3 minor criteria.

MCAS is currently defined by3 criteria: (1) episodic symptoms involving 2 or more organ systems consistent with those produced by sudden release of MC mediators; (2) an increase of the serum tryptase level above baseline values by 20% plus an additional 2 ng/mL; and (3) relief or prevention of attacks by medications that block production of MC mediators or that block their receptors. A subset of patients with mast cell activation (MCA) has more chronic day-to-day symptoms without episodic spells.

Primary MCAS occurs in patients with clonal MC disorders such as SM and in a subset of patients with evidence of an abnormal clone of MC (presence of KIT mutation and/or of the presence of the CD25 marker), but who do not meet the criteria for SM. This latter group is termed monoclonal MCA disorder. Secondary MCAS occurs in patients with IgE-mediated allergic disorders, anaphylaxis, inflammatory, or autoimmune disorders. The third group, idiopathic MCAS, occurs in patients who have no evidence for a clonal MC disorder, or any defined allergic, inflammatory, or autoimmune cause.[1-4]

MC mediators are released during episodes of MCA. Patients may have a history of anaphylaxis in addition to the many other symptoms they experience.[5-7] Some MC mediators are preformed and others are newly synthesized on stimulation.[8-16] These mediators induce the symptoms associated with MC degranulation and activation.[16-21] Serum tryptase, histamine metabolites, prostaglandin (PG) metabolites, and leukotriene (LT) metabolites are validated markers of MCA and degranulation.[22] MCs can also synthesize and secrete numerous other mediators that are not commonly measured in the clinical laboratory and have yet to be incorporated in the definition of SM or MCAS.[23,24]

Tryptase, one of the preformed mediators,[25,26] has a defined diagnostic value for different MC disorders.[27-29] A serum

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No funding was received for this work.
Conflicts of interest: The authors declare that they have no relevant conflicts of interest.
Received for publication October 2, 2019; revised February 18, 2020; accepted for publication February 19, 2020.
Available online ■■
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2213-2198

https://doi.org/10.1016/j.jaip.2020.02.021

*Abbreviations used*

*AA- Arachidonic acid*
*AERD- Aspirin-exacerbated respiratory disease*
*CI- Confidence interval*
*DAO- Diamine oxidase*
*FMLP- N-Formylmethionine-leucyl-phenylalanine*
*HDC- Histidine decarboxylase*
*HNMT- Histamine N-methyltransferase*
*LC-MS/MS- Liquid chromatography-tandem mass spectrometry*
*LT- Leukotriene*
*MC- Mast cell*
*MCA- Mast cell activation*
*MCAS- Mast cell activation syndrome*
*MIAA- N-Methylimidazole acetic acid*
*N-MH- N-Methylhistamine*
*NSAID- Nonsteroidal anti-inflammatory drug*
*PAF- Platelet activating factor*
*PG- Prostaglandin*
*PGDS- Prostaglandin D synthase*
*SM- Systemic mastocytosis*
*$ULTE_4$- Urinary $LTE_4$*

tryptase level above 20 ng/mL is one of the minor diagnostic criteria for SM,[23,30] and an increase in serum tryptase by a minimum of 20% above baseline plus 2 ng/mL is considered a marker of MCA.[1,2,26,31,32] There are a number of disadvantages in relying solely on serum tryptase for diagnosing MCA. Serum tryptase requires a blood sample collection ideally within 1 to 4 hours of a suspected MCA spell. This in turn necessitates an emergency department visit where the test might not be available or offered or where the physician is unwilling to obtain the sample.

Additional approaches to evaluate acute MCA are needed. Assessment of the production of validated urinary MC mediator metabolites offers a supplementary approach.[22] Urine samples can be collected noninvasively by patients at their homes to monitor baseline conditions as well as contemporaneously after a suspected MCA spell. For this purpose a "mail-in" kit can be sent to the patient's home. It contains a urine specimen container, a serum tube for tryptase measurement, and a cold pack for shipping plus mailing instructions. The urine sample is mailed to the laboratory by overnight express for testing. Samples are stable if kept refrigerated or frozen. For sample accrual for acute symptoms, patients are instructed to initially empty the bladder and then to obtain a "fresh" urine specimen as well as a serum tryptase sample between 1 and 4 hours after an episode. Samples are kept refrigerated until mailed by overnight delivery in a container containing a cold pack. These samples supplement the results of tryptase quantitation; however, because of the unreliability of the emergency physician's ordering of tests, notably tryptase, required by patients, the urinary tests do provide measures of 3 of the 4 commonly quantitated MC mediators.

Three validated urinary mediators are currently available for testing in clinical laboratories. They include the histamine metabolite N-methylhistamine (N-MH) (methyl imidazole acetic acid is an available test in Europe but is not offered by any of the major testing labs in the United States), the $PGD_2$ metabolite 2,3-dinor-11$\beta PGF_{2\alpha}$, and the cysteinyl LT metabolite $LTE_4$. One limitation of this approach is the lack of consensus for defining the level of increase in those mediators that would be consistent with an MCA event; however, increased excretions of N-MH and 11$\beta PGF_{2\alpha}$ have been correlated with bone marrow findings in SM and have been helpful in guiding therapy for MCAS (see below). A second concern has been that, although these mediators are mainly produced by MCs, other cellular sources have been reported, albeit often from *in vitro* studies using nonphysiologic stimuli or experimental conditions.

For all of these assays, no dietary limitations are necessary. Avoidance of zileuton is required for baseline assessment of $LTE_4$, and avoidance of aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) is needed for quantitation of baseline levels of 2,3-dinor-11$\beta PGF_{2\alpha}$. In situations in which samples are collected acutely, there is no need or worth of medication avoidance as symptoms are already occurring.

The purposes of this article are to review the available literature regarding the generation and quantitation of those mediators, give some perspective to their production by non-MCs, examine their value in clinical practice, and suggest avenues for research to improve their utility in MC disorders.

## HISTAMINE AND HISTAMINE METABOLITES

Histamine (2-[4-imidazolyl]-ethylamine) is synthesized from L-histidine by histidine decarboxylase (HDC). MCs and basophils each store comparably large amounts of histamine in their secretory granules, whereas other cell types synthesize and secrete histamine, but do not store it intracellularly. MCs and basophils release histamine when they are induced to degranulate.[33,34] Histamine can also be produced by commensal bacteria colonizing mucosal surfaces.[35] Certain foods contain histamine.[36] Although it is frequently stated that certain foods induce nonallergic histamine release, evidence for this is scant.[37,38] Histamine can be a contaminant of spoiled foods as in scombroid poisoning.[39]

Once released, plasma histamine is metabolized rapidly (half-life 1-2 minutes)[40,41] via 2 enzymatic pathways. The first pathway uses histamine N-methyltransferase (HNMT) and the second uses diamine oxidase (DAO). Approximately 70% of histamine is metabolized by HNMT to N-MH, which is then metabolized further by monoamine oxidase to N-methylimidazole acetic acid (MIAA).[34,41] Both N-MH and MIAA can be measured in serum, plasma, and urine. HNMT is found in tissues throughout the body and is particularly important in the central nervous system and bronchial epithelium, where it is the only known histamine metabolizing enzyme.[34] The action of HNMT can be impaired by concurrent use of monoamine oxidase inhibitors.[42]

The second enzymatic pathway uses DAO for histamine catabolism. DAO is a membrane glycoprotein primarily located in the kidney and colon. It is released on stimulation to oxidatively deaminate histamine and other substrates. DAO converts histamine to imidazole acetaldehyde that is then subsequently converted by aldehyde dehydrogenase to imidazole acetic acid and then conjugated with ribose phosphate.[34,43]

### Cells that contain and release histamine

**MCs.** The amount of histamine in MCs has been evaluated in a number of studies. The estimated amount of histamine is 1 to 5.5 pg/cell (lung MCs),[44] 3 to 8 pg/cell in lung, skin, intestinal tissues,[10,45,46] and 3 pg/cell in mucosal MCs.[10] The resting level of histamine in the skin is 5 nM. The release of histamine, $LTC_4$,

**TABLE I.** Histamine content in different human cells

| | Location | Level | Reference |
|---|---|---|---|
| MCs | Lung | 1.5-5 pg/cell | Paterson et al[44] |
| | Lung | 2.5-10 pg/cell | Schulman et al[45] |
| | GI | 3.0 pg/cell | Fox et al[10] |
| | Skin | 4.3 pg/cell | Benyon et al[46] |
| Basophils | | 0.66 pg/cell | Alcaniz et al[49] |
| | | 2.4 pg/cell | Sampson and Archer[48] |
| Platelets | | Baseline 12.2 pmol/$10^9$ platelets<br>39 pmol/$10^9$ after stimulation with PMA | Saxena et al[50] |
| | | 8 ng/$10^9$ platelets | Gill et al[51] |
| Neutrophils | | 0.29 pg/cell | Alcaniz et al[49] |

*GI*, Gastrointestinal; *MC*, mast cell; *PMA*, phorbol myristate acetate.

$PGD_2$, and thromboxane $B_2$ from human lung mast cells or lung fragments is not inhibited after a 24-hour incubation with 1 μM dexamethasone.[47]

**Basophils.** Basophils contain less histamine than do MCs, 0.66 ± 0.0779 pg of histamine/cell.[48] Basophil histamine release is slower (takes 20-30 minutes) than MC histamine release (takes 10 minutes). Incubation of human basophils with dexamethasone for a period of 4 to 24 hours inhibits IgE-dependent histamine release. Steroids, however, do not inhibit histamine release stimulated by other (nonphysiologic) agents f-met peptide (N-Formylmethionine-leucyl-phenylalanine [FMLP]), the phorbol ester 12-O-tetradecanoylphorbol-13-acetate, and the calcium ionophore A23187. In patients with allergies, basophils show approximately 20-fold higher HDC expression (mRNA) than do neutrophils.[49]

**Neutrophils.** Neutrophils do not store histamine, but they contain HDC. HDC induces histamine synthesis and is enzymatically active in neutrophils.[49] A total of 98% of neutrophils from patients with allergies or asthma are positive for HDC versus 10% from healthy donors.[49] Neutrophils from allergic donors exhibit approximately 4-fold higher HCD expression (mRNA) than neutrophils from normal donors.[49] The mean synthesized histamine in neutrophils is approximately 0.29 ± 0.036 pg/cell with 50% of the histamine content released after antigen stimulation (Table I).[49]

**Platelets.** Unstimulated platelets contain small amounts of histamine, approximately 14.7 pmol/$10^9$ platelets, whereas synthesis increases to about 36 pmol (approximately 4.01 ng) of histamine/$10^9$ platelets after stimulation with phorbol myristate acetate, a nonphysiologic activator of protein C kinase. The bulk of histamine remains cytoplasmic.[50] In another report, the amount of histamine in platelets was approximately 8 ng/$10^9$ cells (Table I).[41]

**Other cells.** Eosinophils do not contain HDC and do not release histamine on antigen or lipopolysaccharide stimulation.[49]

### Histamine assays

**Blood histamine.** Plasma samples are better than serum samples for the measurement of blood histamine levels. Serum samples can result in falsely elevated histamine levels as a result of basophil degranulation by complement activation during blood coagulation.[52] The resting level of histamine in plasma is 0.5 to 2 nM.[10] The half-life of intravenously infused histamine in normal volunteers is 1 to 2 minutes, reducing the practicality of this short-lived mediator as a clinically useful biomarker.[41,53] Also, variability of the currently available assays for plasma histamine is problematic, further reducing its reliability as a measure of MC activation.[54]

**Urine histamine and its metabolites.** Normal urinary clearance of histamine is 10 mcg/24 hours. In contrast, urinary histamine clearance in patients with SM is 150 mcg/24 hours. Measurement of urinary histamine is not an accurate measure of MCA. Only a small portion of the histamine that is released into the blood stream appears in the urine. Furthermore, inflammatory conditions in the bladder itself, such as interstitial cystitis, can result in an increased urinary histamine level.[35] The appropriate biomarkers of systemic histamine release from MCs or basophils are the histamine-specific metabolites, N-MH,[55-57] and MIAA.[58-61]

N-MH is obtained from the urine using solid phase extraction. The eluate is subsequently analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS).[62] Either 24-hour urine collections or random "spot" urine samples can be used, as results are expressed as micrograms/gram creatinine (mcg/g creatinine). Normal excretion is age dependent: 0 to 5 years: 120 to 510 mcg/g creatinine; 6 to 16 years: 70 to 330 mcg/g creatinine; >16 years: 30 to 200 mcg/g creatinine (source: Mayo Medical Labs).

## PROSTAGLANDIN $D_2$ ($PGD_2$)

$PGD_2$ is generated from arachidonic acid (AA) by the sequential actions first of either cyclooxygenase 1 or 2, to generate $PGH_2$. $PGH_2$ is subsequently metabolized by either the hemopoietic (H-) or lipocalin (L-) type of PGD synthase (PGDS). L-PGDS is expressed in both the central nervous system and cardiac tissue. H-PGDS is expressed by MCs, megakaryocytes and platelets, monocytes, dendritic cells, $Th_2$ lymphocytes, and lung endothelial cells. H-PGDS is not expressed in basophils.[63]

Large amounts of $PGD_2$ are rapidly synthesized and secreted by MCs activated by FcεRI aggregation. Elevated levels of another PG metabolite, $PGF_2$, were reported during labor.[64] $PGF_2$ is synthesized by the luteal endometrium,[65-67] gestational tissues,[68] human and primate granulosa cells,[69] and hepatocytes.[70] However, there are no data comparing the levels of

**TABLE II.** Prostaglandin $D_2$ production by different human cells

| Cell type | Location and stimulus | Level | Reference |
|---|---|---|---|
| MCs | Human lung, anti-IgE | 50-60 ng/$10^6$ cells | Schleimer et al[72] |
| | Human lung, anti-IgE | 8.6-36.2 ng/$10^6$ cells | Lewis et al[73] |
| | Human intestine, anti-IgE | 21.3 ng/$10^6$ cells | Fox et al[10] |
| | MC cultured from umbilical cord, anti-IgE | 0.7 ng/$10^4$ cells | Obata[79] |
| Basophils | Human, calcium ionophore A23187, anti-IgE, or opsonized zymosan | 0 | van der Donk et al[63] |
| | Human, anti-IgE | ~0.17 ng/$10^6$ cells | Ugajin et al[75] |
| $Th_2$ cells | Human, anti CD3 + anti CD28 | 2-6 ng/$10^6$ cells | Tanaka et al[76] |
| Dendritic cells | Monocyte-derived dendritic cells, calcium ionophore | <1% of the amount produced by MCs | Shimura et al[77] |
| Eosinophils | Sinusitis tissue-derived in AERD, lysyl aspirin | 19.41 ng/$10^6$ cells | Feng et al[78] |

*AERD*, Aspirin-exacerbated respiratory disease; *MC*, mast cell.

PG metabolites produced by MCs and those produced by endometrial tissues.

L-PGDS in brain-endothelial cells from human brain capillaries synthesize $PGD_2$. Both the $PGD_2$ induced by endothelial cells and the exogenous $PGD_2$ added to human brain cell endothelial cultures are converted to 9α,11β-$PGF_2$, a known potent vasoconstrictor.[71]

### Cells that produce and release $PGD_2$

**MCs.** Anti-IgE challenge of human lung mast cells leads to the release of 50 to 60 ng of $PGD_2$ per $10^6$ cells.[72] Skin MCs contain similar amounts. Dexamethasone does not inhibit release of $PGD_2$ from lung fragments or purified MCs that were activated by anti-IgE.[72] Dispersed and concentrated human lung MCs (30%-70% pure) when stimulated with anti-IgE generate 8.6 to 36.2 ng $PGD_2/10^6$ cells.[73] The release of mediators by purified human lung MCs when stimulated with anti-IgE (as percentage of total metabolites) is 56% ± 6.4% for $PGD_2$ versus 21% ± 5.2% for LTC.[74] Airway lumen MCs in bronchoalveolar lavage generate 0.1 to 0.2 pg of $PGD_2$/cell. Umbilical cord MCs produce 0.7 ng/$10^4$ cells with anti-IgE stimulation (Table II). The production of $PGD_2$ by umbilical cord MCs is inhibited by indomethacin and aspirin.[79]

**Basophils.** MCs release the most substantial quantities of $PGD_2$, whereas basophil studies either reported no PG release,[63] or very little $PGD_2$ production at 0.01 ng/$10^6$ basophils after stimulation with anti-IgE.[75]

**$Th_2$ cells.** $PGD_2$ can also be produced by $Th_2$ cells (2-6 ng/$10^6$ cells) but not $Th_1$ cells (Table II).[76] There are no reports of $Th_2$ cell PG secretion in SM or MCAS.

**Dendritic cells.** Dendritic cells have hematopoietic PGDS. Dendritic cells could, therefore, be a source of $PGD_2$ in the skin. However, the amount of $PGD_2$ secreted by dendritic cells is ≤1% of the amount secreted by MCs and 20-fold less than the amount secreted by $Th_2$ cells.[77] Orally ingested niacin, as well as topically applied methylnicotinate to the skin, results in release of $PGD_2$. However, the lack of an accompanying increase of histamine release has suggested that MCs are not the source of $PGD_2$ in these cases.[80,81] This raises the possibility that dendritic cells are a source for $PGD_2$ released in this situation.

**Eosinophils.** Eosinophils from patients with aspirin-exacerbated respiratory disease (AERD) display increased levels of hematopoietic PGDS. This was evaluated by studying gene expression from tissues and blood samples of those patients. The level of PGDS in AERD is much higher than that in asthmatics and other control subjects.[82] Eosinophils purified from sinus tissue of patients with AERD had spontaneous release of increased amounts of $PGD_2$: 1513 pg/$10^5$ cells compared with 826 pg/$10^5$ cells in chronic hyperplastic eosinophilic sinusitis.[82]

## LEUKOTRIENE METABOLITES

$LTC_4$ is generated when AA is converted by 5-lipoxygenase to $LTA_4$. This is followed by the action of $LTC_4$ synthase, which conjugates $LTA_4$ with reduced glutathione to form bioactive $LTC_4$. Bioactive $LTC_4$ is then secreted and rapidly metabolized to $LTD_4$, which is then metabolized to $LTE_4$. $LTC_4$ is produced directly by activated MCs, basophils, eosinophils, dendritic cells, monocytes, and macrophages, and indirectly by transcellular metabolism when $LTA_4$ is transferred from a cell lacking $LTC_4$ synthase to one that has $LTC_4$ synthase such as platelets.[83] $LTE_4$ is used to monitor this pathway in plasma or urine. There is no diurnal variation of $LTE_4$ excretion.[84,85]

### Cells that produce $LTC_4$

**MCs.** $LTC_4$ constituted 21.5% ± 5.2% (anti-IgE) and 17% ± 4.1% (A23187) of total AA metabolites released after overnight incubation of purified human lung MCs with $^3$H-AA and subsequent stimulation.[74] Purified human lung MCs stimulated with anti-IgE release between 28 and 32 × $10^{-11}$ $LTD_4$ mole equivalents per $10^6$ MCs.[86-88] Purified human lung MCs produce over 20-fold more $LTD_4$ mole equivalents than do basophils (Table III).

**Basophils.** LT production by basophils (and eosinophils) is enhanced by preincubation with IL-3 or IL-5. Basophils are capable of generating $LTC_4$ in approximately the same quantities 52.7 ± 25.6 ng/$10^6$ cells as eosinophils (46.5 ± 11.7 ng/$10^6$ cells) (Table III).[89] Basophils rapidly synthesize $LTC_4$ within minutes on allergen binding to specific IgE. IL-5 renders basophils capable of producing large quantities of $LTC_4$ in response to C5a. Without the cytokine, C5a by itself induces only histamine release.[90]

When basophils are pretreated with IL-3 at concentrations of only 0.01 to 1 unit/mL, they become responsive to C3a, releasing large amounts of histamine and also generating LTs.[91]

**Eosinophils.** Normodense eosinophils, maintained in culture with $10^{-11}$ M granulocyte/macrophage colony–stimulating factor, in the presence of 3T3 fibroblasts, become responsive

**TABLE III.** Leukotriene $C_4$ release by different human cells

| Cell | Source; stimulus | Level | Reference |
|---|---|---|---|
| MCs | Human lung MC; anti-IgE | 28-32 $\times$ $10^{-11}$ mole equivalents $LTD_4/10^6$ cells | MacGlashan[86]; Schulman[87]; MacGlashan[88] |
| Basophils | Peripheral blood; anti-IgE | 1.1-1.6 $\times$ $10^{-11}$ mole equivalents $LTD_4/10^6$ cells | Schulman[87]; MacGlashan[88] |
| | Peripheral blood; calcium ionophore | 52.7 $\pm$ 25.6 ng/$10^6$ cells | Mita[89] |
| Eosinophils | Peripheral blood; calcium ionophore | 46.5 $\pm$ 11.7 ng/$10^6$ cells | Mita[89] |
| | Hypodense; FMLP | 26 ng/$10^6$ cells | Owen[92] |
| | Hypereosinophilic donors; calcium ionophore | 69 $\pm$ 28 ng/$10^6$ cells | Weller[93] |
| | Normal donors; calcium ionophore | 38 $\pm$ 3 ng/$10^6$ cells | Weller[93] |
| | Peripheral blood; calcium ionophore | ~30 ng/$10^6$ cells | Owen[94] |

*FMLP*, N-Formylmethionine-leucyl-phenylalanine; *MC*, mast cell.

to transmembrane stimulation with FMLP by day 4 with a maximal effect by day 7. After 7 days of culture, hypodense eosinophils stimulated with 2 $\times$ $10^{-7}$ M FMLP generate 26 ng $LTC_4/10^6$ cells.[92] In another report, eosinophils from hypereosinophilic donors produced 69 $\pm$ 28 ng of $LTC_4/10^6$ cells, whereas eosinophils from normal donors generated 38 $\pm$ 3 ng/$10^6$ cells when stimulated with ionophore A23187 (Table III).[93]

In other studies, purified peripheral blood eosinophils preincubated with cytochalasin B and stimulated in the presence of L-serine with 2.5 μM A23187 produced approximately 30 ng $LTC_4/10^6$ cells, whereas preincubation of cells with FMLP produced a maximum of 8 ng $LTC_4/10^6$ cells.[94]

In addition to the response to calcium ionophore, the production of $LTC_4$ by eosinophils from normal donors when stimulated by naturally occurring soluble agonists has been examined. The stimulants have included FMLP, C5a, and platelet activating factor (PAF). Of these 3 agonists, only FMLP stimulated some production of $LTC_4$ by normal eosinophils. After priming with IL-3 or IL-5, eosinophils produced detectable amounts of $LTC_4$ in response to all 3 agents and generated at least 1 order of magnitude more $LTC_4$ in response to FMLP as compared with stimulation with C5a or PAF.[95]

**Platelets.** Platelets generate $LTC_4$ via transcellular conversion of $LTA_4$ to $LTC_4$ with the percentages of platelet-adherent leukocytes correlating with systemic cysLT production in AERD.[83]

## SUMMARY

Taken together, these studies strongly suggest that, although other sources of these mediators exist, MCs are a major source for histamine, $PGD_2$, and $LTE_4$. In MCAS and SM, where the activity and/or numbers of MC can be greatly increased, the possible contribution of other cell types to significantly affect measured levels of these mediators becomes less credible. Furthermore, increased numbers of these other inflammatory cells in MCAS or SM are not consistently found. Urinary samples, either by 24-hour collection or more recently by random urine specimens, are noninvasive methods for sample accrual for MC mediators because results are currently given as "per" gram or milligram creatinine. Samples obtained contemporaneously with symptoms afford a window of opportunity to document if MC mediator release has occurred and if so which MC mediator(s) are responsible for acute symptoms.

## UTILITY OF URINARY MEDIATORS IN THE DIAGNOSIS AND TREATMENT OF SM AND MCAS

### General comment

A spot urine sample obtained during a period of clinical quiescence should be obtained to serve as a reference point for subsequent samples acquired during times of clinical symptoms. There are currently no dietary limitations necessary before routine urine sampling for mediator quantitation. If possible, avoidance of the following medications for sampling in baseline states includes zileuton (5-lipoxygenase inhibitor affecting levels of $LTC_4$ and excretion of $LTE_4$) and aspirin/NSAIDs (cyclooxygenase inhibitors affecting levels of $PGD_2$ and excretion of 2,3-dinor-11β$PGF_{2\alpha}$).

**Histamine measurements in SM and MCAS.** A high level of histamine in patients with SM is well documented, and in patients with urticaria pigmentosa, both polymyxin B and codeine injected parenterally can cause generalized flushing accompanied by an increase in urinary histamine.[96] However, the plasma histamine level is not a reliable biomarker to screen patients for mastocytosis[97] as this level may be increased in other disorders such as metastatic gastric carcinoid.[98] Rather, measurements of urinary histamine metabolites such as N-MH and MIAA have demonstrated clinical utility in diagnosing SM.[57,99-101] These studies did not report any correlations between elevated urine N-MH levels and symptoms of MCA, perhaps because urine was not reliably collected during the acute period. Urinary excretion of histamine metabolites (NMH or MIAA) has been used in diagnostic algorithms to predict the likelihood of SM in patients lacking skin lesions.[102-104] In one of these studies, receiver operating characteristic curves for tryptase, MIAA, and N-MH were examined. When all patients with a tryptase <10 μg/L were excluded, the areas under the curves for MIAA and N-MH were 0.92 (95% confidence interval [CI], 0.87-0.97) and 0.92 (95% CI, 0.87-0.98), respectively.[104] For patients with documented SM, urinary N-MH was significantly different between c-kit D816V positive and negative patients. N-MH excretion greater than about twice the upper limit of normal (>400 μg/g Cr) corresponded with high degree for the bone marrow presence of atypical MCs, MC aggregates, and c-kit mutation. N-MH excretion also correlated with serum tryptase values.[105]

Treatment of symptoms in SM with antihistamines has shown approximately equal effectiveness of azelastine and chlorpheniramine, though azelastine was superior to chlorpheniramine in suppressing skin responses to histamine and morphine sulfate and in suppressing pruritus.[106] Control of SM symptoms with

cromolyn sodium or the combination of chlorpheniramine and cimetidine is approximately equal.[107] Interestingly, terfenadine and loratadine have been reported to inhibit spontaneous growth of HMC-1 cells, by primary neoplastic MC from human and canine donors, as well as growth by 2 canine MC lines, C2 and NI-1.[108] Ketotifen, reported to have H1 antihistamine and MC stabilizing properties *in vitro*, has been shown to be superior to hydroxyzine for symptomatic control in adult,[109] but not in pediatric mastocytosis.[110] Neither ketotifen nor disodium cromoglycate significantly alters urinary excretion levels of histamine or MIAA.[111]

A study of 25 patients showed that measurement of urinary N-MH was not as useful as measurement of serum tryptase or urinary 11β-$PGF_{2\alpha}$ in diagnosing MCAS. In this study, baseline excretion of N-MH was increased in only 2 patients, both of whom had normal serum tryptase levels and excretion of 11β-$PGF_{2\alpha}$.[112]

**$PGD_2$ in systemic mastocytosis and MCAS.** In 1980, increased $PGD_2$ production was reported in 2 patients one of whom had a bone marrow biopsy consistent with mastocytosis and the second patient with a skin biopsy positive for increased perivascular MCs but with a negative bone marrow biopsy. Although plasma histamine concentrations were significantly increased in both patients during attacks, treatment with antihistamines alone was insufficient to control symptoms. Measured $PGD_2$ production was increased 120-fold above normal in the first patient, who succumbed to an attack, and 18-fold above normal in the second patient. In the second patient, inhibiting $PGD_2$ synthesis with aspirin (3.9 g/day) reduced excretion of the $PGD_2$ metabolite by 80% to 85%, and in addition to use of antihistamine, resulted in symptomatic improvement and prevention of the hypotensive episodes.[113,114] In another study (46 urine samples from 17 biopsy-confirmed patients with SM), the urine $PGD_2$ metabolite PGD-M was increased above normal by as much as 300% in patients in whom excretion of N-methylhistamine was normal.[115] Mean urinary excretion of the $PGD_2$ D-ring metabolite tetranor PGD-M was also significantly higher ($P < .01$) in patients with SM compared with controls (37.2 vs 11.5 ng/mg Cr), with 65% of 17 patients with SM showing elevated levels. The clinical sensitivity of 11β-$PGF_{2\alpha}$ (>1000 ng/24 h) alone for diagnosis of SM was determined to be 53%.[100] Another report demonstrated that 24-hour urinary 11β-$PGF_{2\alpha}$ excretion >3500 ng/day corresponded to a high degree with bone marrow biopsies positive for atypical MCs, the presence of aggregates, the *c-kit* mutation, and correlated with serum tryptase levels.[105]

Aspirin, an inhibitor of cyclooxygenase, has been used to successfully treat symptoms from excessive $PGD_2$ release in SM.[116-119] High doses of aspirin, 3.9 to 5.2 g/day (serum salicylate levels of 20-30 mg/dL), were used by Roberts et al[117,119] to suppress baseline and MC activation–associated $PGD_2$ synthesis. Other studies have also shown the beneficial effect of aspirin (doses 750 mg/day to approximately 4 g/day) in SM.[120,121]

In a study referred to above, of 25 patients with MCAS, only 2 had elevated N-MH values, whereas elevated baseline levels of 24-hour urinary 11β-$PGF_{2\alpha}$ excretion occurred in 17 patients,[112] compared with elevated levels of tryptase in 10 patients. In this study, treatment with aspirin at doses from 81 mg/day to 500 mg BID normalized urinary 11β-$PGF_{2\alpha}$ excretion and controlled symptoms. In another report of patients with MCAS in whom either baseline or episodic release of $PGD_2$ was documented, aspirin (doses of 325 mg/day to 975 mg BID) was effective at preventing symptoms whereas antihistamine treatment alone was ineffective.[122]

Other studies have shown that during anaphylaxis the level of 9α,11β-$PGF_2$ in urine (272 pg/mg Cr) was significantly higher than levels of healthy controls (82 pg/mg Cr).[20] This increase was not observed during bronchial asthma exacerbation (approximately 100 pg/mg Cr). After provocation, 9α,11β-$PGF_2$ increased during the 0- to 3-hour period after challenge. Parallel evaluation of the levels of eosinophil-derived neurotoxin in these patients after anaphylaxis did not reveal any change, thereby suggesting that eosinophils were not the source of this mediator.[20]

**$LTE_4$ in systemic mastocytosis and MCAS.** The mean urinary $LTE_4$ ($ULTE_4$) level in healthy children (103 ± 9 pg/mg Cr) exceeds that of healthy adults (80 ± 7 pg/mg Cr).[123] $ULTE_4$ measurement is a sensitive method to assay total body cysteinyl LT production and changes in production.[124]

Patients with indolent SM both with high intensity of clinical symptoms ($P < .003$) and with low intensity of SM symptoms ($P < .037$) had significantly increased excretion of cysteinyl LTs than controls. Good correlation with urinary N-MH excretion ($r = 0.536$, $P = .005$) was also reported in this study.[125] Compared with a control group of patients with symptoms such as food intolerance, exercise-induced asthma, hives, angioedema, drug allergy, and others, the $ULTE_4$ was significantly elevated in patients with SM and correlated with 24-hour urine N-MH and serum tryptase, but not with urinary 11β-$PGF_{2\alpha}$.[126] The normal $ULTE_4$ measured by LC-MS/MS has been reported to be <104 pg/mg Cr (95th percentile), with a mean value of 97 pg/mg Cr for those with SM versus 50 pg/mg Cr for controls. In this study, the assay was 48% sensitive and 84% specific for SM. Furthermore, when combined with measurements of N-MH and 11β-$PGF_{2\alpha}$, the SM diagnostic sensitivity of the combined mediators increased to 97%.[100]

Quantitation of $ULTE_4$ will likely be useful in the diagnosis of MCAS because studies have also shown increased excretion in anaphylactic reactions.[127] In the report of Ono et al[20] that reported increased production of $PGD_2$ in anaphylaxis, $ULTE_4$ (772 pg/mg Cr) was also significantly higher than levels found in bronchial asthma exacerbation (216 pg/mg Cr) or healthy controls (66 pg/mg Cr). The maximum increase occurred during 3 to 6 hours after anaphylaxis. $ULTE_4$ was higher in those with anaphylaxis who developed severe hypotension (863 pg/mg Cr) versus those without anaphylactic shock (552 pg/mg Cr). Significant correlation was found between the maximum $LTE_4$ and 9α,11β-$PGF_2$ in patients with anaphylactic shock.[20]

In exercise-induced anaphylaxis cases, $LTE_4$ reached a maximum 2 to 5 hours after exercise and returned to normal by hour 20. The maximum increase was 5.5- to 52-fold (median 19-fold $P < .01$). The $LTE_4$ and N-MH rose in parallel during exercise-induced anaphylaxis.[115] This time course of increase and return to normal approximates that reported by Ono et al.[20]

## Areas for clarification and further study

To use urinary mediators in the diagnosis and treatment of MC disorders additional information will be helpful. For example, the minimal rise in urinary histamine, PG, and LT

mediators necessary to define an MCA event is not currently established. Because both MCs and basophils produce histamine, it is possible that both cell types contribute to elevations of this mediator; however, MCs are more numerous than basophils,[128] markedly so in MC disorders, and their histamine content is higher than in basophils, thereby making them the more likely source for histamine increases. However, to separate "MC events" from those that are "basophil events," quantitation of not only serum tryptase but also urinary $PGD_2$ metabolites, neither of which is produced by basophils, could be useful. The contribution of dietary histamine to measured N-MH levels needs to be clarified. For all of these mediators, better knowledge of the time courses of the mediator rise and subsequent decrease could be enhanced by sampling of "spot" urines for MC mediator metabolites at different time points after an episode of MCA. Finally, spot sampling will allow us to better define normal urinary levels of these products in children and to associate specific mediator increases with pediatric MCA events.

## SUMMARY

Although histamine, $PGD_2$, and $LTC_4$ can be produced by other cell types, the MC is the predominant source in disorders with increased MC numbers and/or MC activation. Urinary assays can now be performed on random urine specimens. They are a simple to obtain, noninvasive method to confirm constitutive or episodic release of MC mediators. This capability also serves to guide treatment for blocking mediator production, for example with cyclooxygenase inhibitors (PGs) and zileuton (LTs), or blocking mediator receptors with antihistamines or montelukast. To date, however, as valuable a diagnostic tool as are urinary mediators, these tests have not been incorporated in the definition of either SM or MCAS.

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