Clinical Commentary Review

Nontryptase Urinary and Hematologic Biomarkers of Mast Cell Expansion and Mast Cell Activation: Status 2022

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Learning objectives:

1. To understand that the measured metabolite of prostaglandin (PG)D_2 is also biologically active.

2. To realize that small doses of aspirin are often sufficient to block PGD_2 synthesis.

3. To appreciate that metabolites of leukotriene C_4 (LTC_4), histamine, and PGD_2 are stable when kept refrigerated or frozen.

4. To recognize that commonly used medications can impair the activity of diamine oxidase, an enzyme important for intestinal histamine metabolism.

5. To comprehend that foods processed by bacterial fermentation, spoiled foods (scombroid poisoning), and carcinoid tumors can be sources of histamine in addition to the histamine stored in the granules of mast cells and basophils.

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Quantitation of urinary metabolites of histamine, prostaglandin D_2, and leukotriene E_4 can fill the gap in our current efforts to improve diagnosis and management of symptomatic patients with systemic mastocytosis, and/or mast cell activation syndrome, In addition, patients symptomatic due to mast cell activation but who do not meet all the criteria for mast cell activation syndrome can have elevated baseline mediator metabolites. Serum tryptase levels have been the workhorse in diagnosing these disorders, but it has several drawbacks including the need to obtain acute and baseline samples, which require 2 visits to health care facilities and 2 venipunctures. Recently, increased baseline tryptase level has been reported in hereditary alpha tryptasemia, complicating diagnostic possibilities of an increased baseline tryptase level. Furthermore, no treatment can specifically be targeted at tryptase itself. In contrast, the finding of 1 or more elevated urinary levels of histamine, prostaglandin D_2, and/or leukotriene E_4 metabolites (1) greatly narrows diagnostic possibilities for causes of symptoms; (2) informs the practitioner what specific metabolic pathways are involved; and (3) targets the treatment in a specific, direct fashion. As a bonus, baseline spot/random urine samples can be obtained by the patients themselves and repeated at exactly the correct time when symptoms occur. © **2022 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2022;10:1974-84)**

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Abbreviations used
cys-LT- cysteinyl leukotriene
ISM- indolent SM
LT- leukotriene
MC- mast cell
MCA- mast cell activation
MCAS- mast cell activation syndrome
MIAA- methylimidazole acetic acid
N-MH- N*-methyl histamine*
PG- prostaglandin
PGD-M- 9α,11β-dihydroxy-15-oxo-2,3,18,19-tetranorprost-5-ene-1,20-dioic acid
SM- systemic mastocytosis
ULTE$_4$- urinary LTE$_4$

INTRODUCTION

With thousands of patients currently experiencing chronic and/or episodic symptoms ascribed, either correctly or incorrectly, to systemic mastocytosis (SM) or mast cell activation syndrome (MCAS), the need has never been greater to feel confident in our ability to correctly diagnose and treat symptoms of these disorders caused by excessive levels of mast cell (MC) mediators. The discovery of biomarkers that (1) provide evidence for the pathophysiology of symptoms, (2) allow targeted, preventive therapy, and (3) distinguish these disorders from other maladies with similar symptoms is a goal worth pursuing. The first use of the term "mastocytosis" appeared in 1947.[1,2] This predated by over 3 decades the term "mast cell activation" (MCA), which appeared in a report of physical urticarias and angioedema in 1980[3] and by 45 years the first use in 1992 of the idiom "mast cell activation syndrome."[4]

In addition to serum tryptase, MCs are sources of several mediators that can be used to diagnose and treat SM and MCAS. These include prostaglandin (PG)D_2, histamine, and leukotriene(LT)C_4.[5] However, at present, testing for metabolites of these mediators remains underused possibly because large cohort studies validating blood or urinary levels of nontryptase mediators or their metabolites as criteria for diagnosis have not been published.

MCs can generate large amounts of PGD_2[6] and are the predominant source of this mediator, which is not produced by basophils.[7] Although MCs and basophils both synthesize histamine, which is stored in cytoplasmic granules, ready for immediate release, the histamine content of MCs is higher than that of basophils.[8,9] In SM and MCAS where MC numbers and/or activation are increased, it is reasonable to presuppose that MCs account for most of the released histamine and, subsequently measured, histamine metabolites. Furthermore, in clinical scenarios in which measurements of tryptase plus urinary metabolite(s) of PGD_2 are concurrently increased, any parallel measured increase in histamine is likely also MC-derived.

It must be recognized that eosinophils, in addition to MCs, are capable of producing PGD_2 as well as cysteinyl leukotrienes (cys-LTs).[10] Among their effects, cys-LTs inhibit eosinophil apoptosis, and prolong eosinophil survival.[10] Yet, clinical conditions in which eosinophils have been documented to be an important source of PGD_2 or cys-LTs are easily distinguished from SM and MCAS.[11,12] To date, metabolites of PGD_2, histamine, and LTC_4 have not been incorporated into the criteria used to diagnose SM or MCAS, although there are numerous reports of using these measurements to guide the treatment of SM and MCAS, including narratives predating the discovery of tryptase.[13]

MCs are the source of many products including prestored mediators (biogenic amines, enzymes, growth factors, peptides, proteoglycans) as well as those synthesized *de novo* (chemokines, cytokines, nitric oxide, and phospholipid metabolites).[14] These products have their own physiological activities but additionally affect other cell types such as eosinophils, which in turn release their own products.[15] The potential contribution(s) of the preponderance of these MC products vis-a vis SM or MCAS-associated symptoms remains largely unsubstantiated. Baseline and symptom-associated levels, and/or physiological effects resulting from their direct administration/infusion, are also largely lacking. Table I provides a list of common SM and MCAS symptoms from recent reviews[16] (P. Valent, K. Hartmann, P. Bonadonna, T. Gullen, K. Brockow, I. Alvarez-Twose, et al, Global classification of mast cell activation disorders An ICD-10-Adjusted proposal of the ECNM-AIM Consortium, submitted 2022) along with potential or documented MC mediator associations and where possible known physiologic effects of these mediators that have been documented in other systems.

When considering symptoms in SM and MCAS it is also important to recognize that MCs are heterogeneous. Phenotypic heterogeneity of MCs, that is, MC subsets containing only tryptase and those containing tryptase and chymase differ in their proportions in tissues, in their mediator and cytokine content, and in factors that trigger their release. In addition, the relative proportions of MC subsets can vary with tissue inflammation and fibrosis. This variability could influence clinical symptoms.[17]

MC triggers that cause release of mediators but do not cause MC degranulation (corticotrophin-releasing hormone, stem cell factor, cytokines [IL-33, IL-1β], heavy metals [mercury, aluminum, cadmium], herbicides, certain pathogens)[14] are also recognized and recently, a new receptor for MCA, Mas-related G protein—coupled receptor X2, chiefly found in skin MCs, where MC subsets containing tryptase and chymase predominate, has been described. Direct activation of this receptor by drugs such as vancomycin, atracurium, morphine, ciprofloxacin, and others may lead to non—IgE-mediated MC activation. However, drug concentrations required to trigger Mas-related G protein—coupled receptor X2 are generally very high.[18,19] The importance of a great many of these triggers and released MC mediators in the clinical setting awaits validation.

MC MEDIATORS AND THEIR METABOLITES IN THE EVALUATION OF MCAS AND SM

Despite the large number of MC-synthesized mediators, nontryptase biomarkers used for assessment of SM and MCAS presently include only the metabolites of the prestored amine, histamine, and the *de novo* synthesized phospholipids PGD_2 and LTC_4. Currently, these markers are not included as criteria in the diagnosis of SM or MCAS. However, consensus groups have advocated the use of increases above measured baseline levels of these metabolites as potential diagnostic criteria for MCAS events when serum tryptase levels are not available or are inconclusive.[20,21]

TABLE I. MC-specific and related mediators and their possible clinical effects in SM and MCAS

Symptoms of MCAS	Mediator(s)	Comment/possible mechanism or known physiologic effect(s)
Abdominal/ gastrointestinal		
Cramping/abdominal pain	Histamine	Increased gastric acid production, increased venular permeability
	2,3 dinor-11β-$PGF_2\alpha$	Contraction of uterine smooth muscle
	Tryptase	Contraction of uterine smooth muscle
	Chymase	Intestinal smooth muscle contraction
Nausea, vomiting, diarrhea		
	Histamine	Increased venular permeability
	Chymase	Intestinal smooth muscle contraction; increased gastric acid secretion
	Tryptase	Contraction of uterine smooth muscle
	IL-6	Intestinal smooth muscle contraction; increased gastric acid secretion
	PGD_2	Increased venular permeability
		Increased intestinal permeability (in IBS); secretory diarrhea associated with PGD_2 overproduction/antihistamine resistance
		Levels higher in diarrhea-prone IBS; contraction of colonic smooth muscle
Dermatologic		
(dry)Flushing	Histamine	Increased venular permeability
	PGD_2	Increased venular permeability; niacin-induced flushing is associated with release of PGD_2 from skin langerhans cells
Pruritus	Histamine	Reciprocal interaction between MCs and sensory nerves; stimulation of mechano-insensitive C-fibers in the skin
	FGF	Vasodilation
Edema	PGD_2	Edema is induced by infusions of PGD_2 16-128 ng/kg/min
Urticaria/angioedema		
	Histamine	Increased vascular permeability; vasodilation; release in cold urticaria documented
	PGD_2	Increased vascular permeability
	VEGF	Increased vascular permeability
	cys-LTs	Increased vascular permeability
Neurologic/psychiatric		
Headache	Histamine	Known cause of vascular headaches for >100 y; increased central histamine causes headache
	TNF-α	Infusion of 0.5 μg/kg/min × 20 min induces headache; endogenous formation of NO in intracranial arteries
	IL-6	Infusion 384 ng/kg/min over 25 min caused cephalic arterial dilation and mild headache
	2,3 dinor-11β-$PGF_2\alpha$	Increased levels found in patients with tension headache
Fatigue	IL-6	Reduces fatigue-induction of sleep
Depression	IL-6	Increased levels reported in patients with chronic fatigue syndrome
	PGD_2	Fatigue and depression in patients with chronic renal failure on hemodialysis; cyclooxygenase-2 inhibition reported to improve depression
Seizures	IL-6	Reduces fatigue-induction of sleep; epileptic seizures induce production of IL-6
Psychosis	Histamine	Benefit of histamine 2 receptor blockade reported in schizophrenia
Cardiovascular		
Hypotension/syncope	PGD_2	Vasodilation at low doses
	Histamine	Decreased diastolic blood pressure with infusion at rates of 16, 32, 64, 96, and 128 ng/kg/min
Anaphylaxis	PAF	Activation of kinases, release of prostaglandins from vascular smooth muscle cells; bronchoconstriction; increased vascular permeability; ?increased NO synthesis
	Histamine	Vasodilatation, increased vascular permeability, increased heart rate, increased cardiac contraction, and increased glandular secretion
	Tryptase	Tryptase activation of the contact (kallikrein-kinin) system
	PGD_2	Bronchoconstriction, peripheral vasodilation, coronary and pulmonary artery vasoconstriction; enhances histamine release from basophils
	cys-LTs	Bronchoconstriction; increased vascular permeability
Hypertension	PGD_2	Vasoconstriction at high doses
	Chymase	Chymase-dependent generation of angiotensin 2 in human atrial tissue
	TNF-α	Endothelium-dependent vasodilation is impaired by TNF-α in an NO-dependent manner

(continued)

TABLE I. *(Continued)*

Symptoms of MCAS	Mediator(s)	Comment/possible mechanism or known physiologic effect(s)
Tachycardia	PGD_2	Small but statistically significant tachycardia found at PGD_2 infusion rates of 64 and 128 ng/kg/min
	Histamine	Dose-dependent increased heart rate with infusion at rates of 16, 32, 64, 96, and 128 ng/kg/min
Chest pain	cys-LTs	Increased LTE_4 excretion accompanied chest pain/myocardial ischemia in patients with AERD; symptoms resolved with zileuton use
	Chymase	Chymase-dependent generation of angiotensin 2 in human atrial tissue
	Histamine	Coronary artery vasospasm
	PGD_2	PGD_2 is transformed by coronary arteries to 2,3 dinor-11B-$PGF_2\alpha$, which contracts human coronary artery rings
Respiratory		
Wheezing/asthma/dyspnea	Histamine	Bronchial smooth muscle contraction; increased airway mucus production
	cys-LTs	Bronchial smooth muscle contraction; increased airway mucus production
	IL-13	Contraction of bronchial airways; induces tissue inflammation, mucus hyperproduction, goblet cell hyperplasia, subepithelial airway fibrosis, Charcot-Leyden–like crystal deposition, airways obstruction, and airway hyper-responsiveness on methacholine challenge
	Chymase	Constriction of airways
	Basic-fgf	Contraction of bronchial smooth muscle; airway remodeling, smooth muscle proliferation
	PAF	Proliferation of airway smooth muscle; PAF-mediated pulmonary edema reported in animal models
Stridor	cys-LTs	Mucus hypersecretion; nonspecific bronchial hyperreactivity
Naso-ocular		
Nasal congestion	Histamine	Increased vascular permeability
	IL-13	Important in late-phase allergic response
	PGD_2	Increased vascular permeability; mucus hypersecretion
Nasal pruritus	Histamine	Activation of mechanically insensitive C-fibers that transmit itch to spinal cord
Conjunctival injection	Histamine	Levels increased in tears of patients with allergic conjunctivitis
	LTC_4	Levels increased in tears of patients with allergic conjunctivitis
Hematologic		
Bleeding (in advanced SM)	Tryptase	Alpha chain of fibrinogen susceptible to degradation by human B tryptase; tryptase-heparin complexes inhibit generation of fibrin via proteolytic destruction of fibrinogen
	VEGF	Anticoagulation—binding to and activation of AT III–> inactivation of thrombin, factor Xa
	PGD_2	Inhibition of platelet aggregation
	2,3 dinor-11β-$PGF_2\alpha$	Inhibition of platelet aggregation
	Heparin	Binding to and activation of antithrombin III, inhibition of thrombin and factor Xa
Miscellaneous		
Constitutional (fever, weight loss, loss of appetite, myalgias)	TNF-α	Cachexia; synthesis of acute-phase proteins; biomarker of inflammation
	IL-6	Involved in cachexia progression in some cancers
Osteoporosis	Histamine	Stimulatory effect on osteoclasts and their precursors
	TNF-α	Promotes osteoclast formation; inhibits osteoblast activity
	IL-6	Promotes osteoclast formation
	Stem cell factor	Modulation of osteoclast activity
	RANKL, osteoprotegerin	MCs are sources of both RANKL and osteoprotegerin
	Cytokines (IL-1, IL-3, TGF-β)	These cytokines are known to promote osteoclastic formation in a number of model systems
	Heparin	Binds to osteoprotegerin, preventing interaction with RANKL, promoting RANK/RANKL interaction & activation of osteoclasts

AERD, Aspirin-exacerbated respiratory disease; *fgf*, fibroblast growth factor; *IBS*, irritable bowel syndrome; *PAF*, platelet-activating factor; *RANKL*, receptor activator of nuclear factor kappa beta; *VEGF*, vascular endothelial growth factor.

Prostaglandin D_2

Generation and metabolism of PGD_2; Assays for PGD_2 metabolites. Not synthesized by basophils, PGD_2, along with tryptase, is primarily an MC product.[7,15] PGD_2 is derived from arachidonic acid by the sequential actions of cyclooxygenases 1 and 2 to generate PGH_2, the common precursor for PGs and thromboxanes, followed by the actions of either hematopoietic or lipocalin PGD synthase.

TABLE II. Comparison of blood testing and urine testing to monitor MC mediators

Blood testing
1. For tryptase testing, 2 venipunctures are needed: event-related and baseline
2. Patient must travel to an emergency room, physician's office, or clinic
3. Emergency room physician may not be willing to draw the sample or give some other reason for not doing the test
4. Plasma histamine assay requires an additional plasma EDTA sample that must be cooled immediately and then frozen. Plasma histamine levels have a circadian rhythm and are not useful to screen patients for SM
Urine tests
1. Sample collection is easily accomplished by the patient for either routine monitoring or immediately following an attack to check for mediator spikes
a. For event-related samples, empty the bladder and within 4 h of the attack collect a random specimen of fresh urine
b. Samples are stable for 7 d when kept refrigerated. Mail the sample back to the lab by overnight express using the insulated container and enclosed cold pack. A frozen sample is not necessary
2. Noninvasive, sensitive
3. Monitors metabolites of 3 MC mediators

EDTA, Ethylenediamine tetraacetic acid.

PGD_2 is subsequently metabolized by 3 pathways to give J-ring (15 deoxyΔ12,14-PDJ_2), D-ring (tetranor PGDM), and F-ring metabolites (11β-$PGF_2\alpha$; 2,3 dinor-11β-$PGF_2\alpha$; 9α,11β-dihydroxy-15-oxo-2,3,18,19-tetranorprost-5-ene-1,20-dioic acid) (PGD-M).[22,23] Inhibition of cyclooxygenase-1 but not selective inhibition of cyclooxygenase-2 depressed both PGD-M and 2,3-dinor-11β-PGF2α excretion.[23]

Plasma 9α,11β-PGF_2 (6 pg/mL), its 24-hour urinary excretion (982 ng/24 h) in a normal volunteer, and increased urinary excretion (6634 ng/24 h) in a patient with SM were reported in 1985.[24] Currently, the urinary levels of the F-ring metabolites 11β-$PGF_2\alpha$ and 2,3 dinor-11β-$PGF_2\alpha$ are assayed by liquid chromatography followed by tandem mass spectroscopy (LC-MS/MS) (current reference range for 2,3 dinor-11β-$PGF_2\alpha$, <1802 pg/mg Cr [Source: Mayo Medical Labs, Rochester, MN]). In healthy volunteers and in aspirin-tolerant patients with asthma, no circadian changes have been reported in the urinary 9α,11β-PGF_2 level.[25] Elevated urinary levels of the PGD_2 D-ring metabolite tetranor PGDM quantified by enzyme immunoassay are reported in SM.[26]

For routine measurements or to sample contemporaneously with a suspected MCA "attack," a random urine specimen is sufficient. We recommend obtaining a sample of fresh urine during the first 2 to 4 hours following an attack.

In previous reports, urine samples have detected marked increases in 1 or more of the MC metabolites of PGD_2 within a time frame of between 2 and 5 hours after MCAS episodes including anaphylaxis,[27-31] after aspirin provocation in aspirin-induced asthma,[32] and after mannitol inhalation in patients with asthma.[33] A comparison of blood and urine testing for MC mediator metabolites is presented in Table II. Table III presents that MC mediator metabolites are stable for at least 7 days when samples are refrigerated, and for at least 2 weeks when frozen. For comparison, tryptase values are stable for 72 hours at room temperature and for 7 days when stored at 4°C.[34]

Biologic effects of PGD_2. The biologic effects of PGD_2 cause many of the patient-reported symptoms in SM and MCAS and are mediated via activation of D prostanoid receptors DP1 and DP2.[35-37] 9α,11β-PGF_2, the initial F-ring product of PGD_2 metabolism by an 11-keto reductase enzyme, is itself biologically active with effects reported in several species.[16] The stability of urinary mediators stored at different temperatures is presented in Table III.

PGD_2 in SM and MCAS. Human lung MCs release large amounts of PGD_2 (up to 60 ng $PGD_2/10^6$ cells stimulated with anti-IgE). This effect is not inhibited by dexamethasone.[38,39]

In 1980, before the discovery of tryptase, the clinical utility of measuring PGD_2 metabolites was documented in 2 patients diagnosed with SM. Both patients had symptoms of MCA and elevated excretion of histamine; however, treatment with antihistamines was ineffective at preventing or treating acute attacks. Urinary samples from both patients revealed high levels of the PGD_2 metabolite 9α-hydroxy-11,15-dioxo-2,3,4,5-tetranorprostane-1,20-dioic acid. Production of PGD_2 was increased 18-fold above normal in patient 2 and 120-fold above normal in patient 1 who succumbed to an attack. Addition of aspirin (975 mg 4 times daily) to the antihistamine program resulted in a decreased excretion of this PGD_2 metabolite by 80% to 85% and a cessation of flushing and hypotension in patient 2.[13]

Following the discovery that aspirin inhibits nicotinic acid–induced flushing,[40] subsequent reports documented contemporaneous flush-associated increased plasma levels of 9α,11β-PGF_2 in these patients.[41] The skin was identified as the major site of PGD_2 release after topical application of methyl-nicotinate (10^{-1} mol) with plasma levels of 9α,11β-PGF_2 rising 25- to 33-fold.[42] PGD_2 (and histamine) release was also documented in cold urticaria reactions.[43]

The effectiveness of aspirin therapy to prevent the actions of PGD_2 became generally acknowledged, thereby reinforcing the credibility of PGD_2 as a biomarker important in the pathophysiology of symptoms. Aspirin doses in several of these studies were relatively high, up to 4 g/d.[28] A later survey of aspirin use in 20 patients with SM confirmed that much lower doses of aspirin (generally 81-325 mg twice a day) were sufficient to reduce elevated 24-hour excretion levels of urinary 11β-$PGF_2\alpha$ to normal in most patients with SM.[44]

Although no fatalities from aspirin ingestion have been reported in SM or MCAS, it was recognized in earlier and subsequent reports that aspirin was capable of triggering MC degranulation often with severe associated symptoms.[13,45-47] This side effect occurs in 5% to 10% or less of patients with SM.[44] A recent study shows that most patients with SM tolerate aspirin without difficulty.[48] Nevertheless, chronic aspirin use can lead to serious gastrointestinal side effects. Therefore, to minimize this risk, several measures can be taken including the use of enteric-coated aspirin preparations taken on a full stomach, plus a regular H_2 receptor blocker or proton pump inhibitor. Graded dosing with aspirin can often be successful when aspirin is required to block PG synthesis in a patient with a history of aspirin intolerance.[49]

SM and MCAS. Elevated levels of PGD_2 are present in SM and at times of MCAS as reflected by urinary as well as plasma measurements of PGD_2 metabolites: (1) Measurement of urinary

TABLE III. Temperature-dependent stability of urinary MC mediator metabolites

Metabolite	Ambient (room temperature)	Refrigerated	Frozen
N-MH	24 h	8 d	14 d
LTE_4	24 h	7 d	30 d
2,3 dinor 11β-$PGF_2\alpha$	24 h	14 d	30 d

PGD-M (46 urine samples from 17 biopsy-confirmed patients with SM) demonstrated that these levels are increased to a greater degree than are levels of *N*-methyl histamine (N-MH). In 4 patients with normal excretion of N-MH, the excretion of PGD-M was increased above normal by up to 300 %.[50] (2) In patients with SM, 24-hour urinary excretion of 11β-$PGF_2\alpha$ of more than 3500 ng corresponded with a high degree of bone marrow biopsies positive for atypical MCs, the presence of MC aggregates. There was a significant positive correlation between 24-hour urinary excretion of 11β-$PGF_2\alpha$ and serum tryptase values ($P = .0015$).[51] (3) One report detailed the results in 4 patients with MCAS and bone marrow biopsies negative for SM. Increased baseline urinary excretion of 11β-$PGF_2\alpha$ was present in 2 patients. For the remaining 2 patients, baseline levels of urinary 11β-$PGF_2\alpha$ and N-MH were normal, but during acute symptoms, the excretion only of 11β-$PGF_2\alpha$ increased markedly. Treatment with aspirin (range, 325 mg/d-975 mg twice a day) normalized elevated baseline excretion of 11β-$PGF_2\alpha$ and prevented symptoms in all 4 patients.[52] (4) In a report featuring 25 patients with MCAS, baseline levels of 11β-$PGF_2\alpha$ were increased in 17 patients, greatly exceeding the frequency of elevated baseline tryptase values (10 patients) or urinary N-MH (2 patients).[53] (5) When MC mediator metabolites were measured in patients presenting with symptoms of MCA, a solitary baseline or symptom-associated increase in 11β-$PGF_2\alpha$ was found in 57% of patients. This exceeded the percentage of patients with increased values of tryptase (29%), urinary LTE_4 (26%), or N-MH (3.9%).[54]

Plasma levels of PGD_2 metabolites. Plasma levels of PGD-M as well as 11β-$PGF_2\alpha$ are elevated in patients with quiescent SM, and during a fatal attack of MCAS, plasma levels of PGD-M increased more than 10,000-fold.[55] Following niacin ingestion, both PGD-M and 11β-$PGF_2\alpha$ increased. Plasma levels of 11β-$PGF_2\alpha$ peaked between 30 and 45 minutes and returned to baseline by 2 hours. In contrast, plasma levels of PGD-M reached a maximum after 1 to 3 hours and remained elevated above normal even after 7 to 7.5 hours.[55]

Histamine

Histamine 2-[4-imidazolyl]-ethylamine, a biogenic amine produced by the action of histidine decarboxylase on the essential amino acid histidine, is stored in the granules only of MCs and basophils. The histamine content of MCs varies with the tissue from which MCs are purified. MCs isolated from the lung have up to 10 pg/cell,[56,57] MCs from the gastrointestinal tract 3.0 pg/cell,[58] and MCs from the skin 4.3 pg/cell.[59] The histamine content of basophils is lower (0.66-2.4 pg/cell) than that of MCs.[8,9]

Sources of histamine and triggers for its release. - Sources of histamine in addition to that stored in MCs and basophils have the potential to contribute to clinical symptoms and to the measured levels of histamine metabolites. Common examples include the production of histamine by commensal bacteria colonizing mucosal surfaces,[60] and the histamine content of foods either as a natural occurrence,[61] or as the result of spoilage.[62] Histamine-producing gastric carcinoid tumors have also been reported.[63] Although cross-linking of $Fc_\varepsilon R1$ receptors plays a prominent role in triggering MC degranulation, there exist a great many other triggers such as stress,[64] neuropeptides, $C3_a$ and $C5_a$, cytokines, hyperosmolarity, lipoproteins, adenosine, superoxides, hypoxia, and chemical and physical factors including stem cell factor.[65-67]

Physiologic actions and related symptoms from histamine. The known physiologic actions of histamine in allergic inflammation are related to the types and locations of histamine receptors in the body. Symptoms from stimulation of H_1 receptors include pruritus, pain, vasodilation, increased vascular permeability causing nasal secretion from plasma leakage, hypotension, flushing, headache, tachycardia, stimulation of vagal afferent nerves, and cough receptors.[68] Symptoms that result from stimulation of H_2 receptors include increased gastric acid secretion, diarrhea, increased vascular permeability, hypotension, flushing, headache, tachycardia, chronotropic and inotropic activity, bronchodilation, and mucus production.[68,69]

Histamine metabolism. Factors that impair the metabolism of histamine must be considered when evaluating the potential contribution of histamine to acute or ongoing symptoms and its utility as a biomarker. About 70% of histamine is metabolized by histamine *N*-methyl transferase, which catalyzes the transfer of a methyl group from *S*-adenosyl-L-methionine to histamine yielding N-MH. N-MH is subsequently metabolized by monoamine oxidase to *M*-methylimidazole acetic acid (MIAA). N-MH and MIAA both can be measured in serum, plasma, and urine.[70,71]

Following solid-phase extraction, N-MH is measured by LC-MS/MS.[72] The excretion of N-MH in normal volunteers is highest in children (120-510 μg/g Cr, 0-5 years; 70-320 μg/g Cr, 6-16 years) and is lowest for those older than 16 years (30-200 μg/g Cr) (Source: Mayo Medical Labs). A number of medications including diphenhydramine, amodiaquine, metoprine, and tacrine are potent inhibitors of histamine *N*-methyl transferase.[73] In a cohort of nonatopic children, no influence of food intake or circadian rhythm affected the urinary excretion of histamine or N-MH.

Diamine oxidase, the second histamine-metabolizing enzyme, is present in the kidney and colon and oxidatively deaminates histamine to imidazole acetaldehyde, which is subsequently converted by aldehyde dehydrogenase to imidazole acetic acid and then conjugated with ribose phosphate.[70,74] Commonly used medications such as cimetidine,[75] clavulanic acid,[76] and metformin[77] can inhibit the activity of intestinal diamine oxidase, as can many others.[78] Table IV summarizes sources of histamine and factors that potentially influence measured levels of histamine metabolites.

TABLE IV. Sources of histamine and factors that potentially influence histamine metabolism

Histamine sources
MC and basophil degranulation
Enterochromaffin-like cells in the gastric mucosa
Gastric neuroendocrine tumors (carcinoid tumors)
Foods in which microbial fermentation processes are involved. Eg, sauerkraut, aged cheezes, red wine, tofu, processed meats
Spoiled foods (scombroid poisoning)
Foods without increased histamine content, considered to possibly cause release of histamine: tomatoes, nuts, chocolate, egg whites
Factors that influence histamine metabolism
DAO inhibitors: Eg, red wine, cimetidine, metoclopramide, amiloride
Histamine-N-MT inhibitors: Eg, diphenhydramine, tacrine, metoprine, amodiaquine
MAO-B Inhibitors: Eg, selegiline
Diurnal variation of plasma histamine levels

DAO, Diamine oxidase; *MAO-B*, monoamine oxidase-B; *N-MT*, *N-methyl* transferase.

The problems inherent in using blood histamine levels as biomarkers. Plasma histamine samples are more reliable than serum samples for the measurement of blood histamine levels because the collection of serum samples may result in a false elevation of histamine due to complement activation during blood coagulation.[79] The half- life of intravenously infused histamine in normal volunteers is only 1 to 2 minutes,[80] making the analysis of plasma levels of histamine impractical in routine clinical settings.

Baseline plasma histamine levels were not useful to screen patients for SM. Thirty percent of the patients with indolent (I) SM had a normal initial plasma histamine level. Furthermore, a circadian pattern of plasma histamine levels was found in patients with SM, with highest levels around 2:00 AM and lowest values at approximately 2:00 PM.[81] The circadian pattern of *plasma* histamine levels has also been found in other groups including normal volunteers, and diurnal variation in *urinary* histamine levels has been reported both in patients with asthma and in normal volunteers.[82,83]

Urinary histamine metabolites as biomarkers. Assays for both MIAA and N-MH have been used to evaluate patients with SM and MCAS. Excretion of urinary MIAA, but not urinary histamine itself, has a significant correlation with the extent of MC infiltration in skin and internal organs.[84,85] It has also been demonstrated that in both 24-hour collections and random urine specimen, N-MH and N-MIAA were more specific and sensitive for diagnosing SM than was determination of histamine.[86] There is also a positive correlation between the excretion of MIAA and MC numbers in bone marrow sections.[87,88]

Urinary histamine metabolite excretion in combination with serum tryptase levels has been incorporated into an algorithm used to predict ISM in patients lacking skin lesions but having symptoms of MCA. With tryptase values exceeding 10 μg/L, the risk was low if MIAA and N-MH levels were normal and high if these values were elevated.[89] N-MH excretion was shown to be significantly higher in patients with SM positive for the *c-kit* Asp816Val mutation than in those negative for the mutation. Excretion of N-MH greater than 400 μg/g Cr corresponded with high degree for bone marrow presence of atypical MCs and MC aggregates, and the N-MH excretion was statistically higher in patients having the *c-kit* D816V mutation compared with the group lacking the mutation. The N-MH excretion also positively correlated with serum tryptase values.[51]

In contrast, measurement of baseline urinary N-MH excretion has not been helpful to identify patients with MCAS or with MCA. In a series of 25 patients, only 2 had increased N-MH excretion.[55] Likewise, in a second report of MC mediator levels from patients presenting with symptoms of MCA, among 52 patients who were found to have a single mediator increased, only 2 had increased excretion of N-MH.[54] In another report, increased levels of N-MH excretion were found in only 18% of patients with MCA compared with 76% of patients with ISM.[90] Similarly, a study of 275 patients showed that the sensitivity of N-MH was only 22% for MCAS, whereas for SM this rose to 43%. In this study, the sensitivity of baseline serum tryptase was 10% for MCAS and 73% for SM.[91]

Leukotriene E_4

Following the action of 5-lipoxygenase/5-lipoxygenase–activating protein on arachidonic acid to generate LTA_4, LTC_4 is generated by the action of LTC_4 synthase, which conjugates LTA_4 with glutathione to form bioactive LTC_4. This compound is then secreted and rapidly metabolized to LTD_4 and then to LTE_4, the stable metabolite used to monitor this pathway in plasma or urine. In contrast to plasma histamine,[81] there is no diurnal variation in the excretion of LTE_4.[92,93] MCs, basophils, eosinophils, dendritic cells, monocytes, and macrophages can synthesize LTC_4. Platelets and other cells with LTC_4 synthase activity can convert LTA_4 derived from extracellular sources to LTC_4, a process termed transcellular conversion.[94-96] Although basophils also produce LTC_4, purified human lung anti–IgE-stimulated MCs produce more than 20-fold more LTD_4 equivalents than do anti–IgE-stimulated basophils.[97-99]

Physiologic activities of cys-LTs. Diverse physiological effects from cys-LTs that can contribute to symptoms common to SM, MCAS, and MCA have been documented in various species. The effects most relevant to symptoms include increased vasopermeability, dermal edema, coronary vasoconstriction, bronchoconstriction, airway remodeling and smooth muscle proliferation (asthma), eosinophil chemotaxis and reduction of eosinophil apoptosis, and synergism with other cytokines to increase proliferation of eosinophils.[10,100]

Measurement of LTE_4. LTE_4 is measured by LC-MS/MS, with a normal urinary LTE_4 ($ULTE_4$) level defined as less than 104 pg/mg Cr (95th per centile). Normal volunteers have a mean $ULTE_4$ of 50 pg/mg Cr compared with a value of 97 pg/mg Cr for those with SM. The $ULTE_4$ was found to be 48% sensitive and 84% specific for SM, and the combination of N-MH, 2,3 dinor-11β-$PGF_2\alpha$, and LTE_4 measurements was 97% sensitive for SM with a specificity of 61%.[101] $ULTE_4$ levels increase substantially when patients with SM are given aspirin to reduce PGD_2 synthesis.[102] The reason(s) for this occurrence is unknown.

TABLE V. Baseline and MCAS-associated urinary MC mediator metabolites and serum tryptase changes in a patient evaluated for "spells"

Mediator (reference range)	Baseline	Following attack	% change
Tryptase (<11.5 ng/mL)	4.7	8.7	85
2,3 dinor 11β-$PGF_2\alpha$ (<5205 pg/mg Cr)	3052	13,718	350
N-MH (<200 mcg/g Cr)	132	194	47
LTE_4 (<104 pg/mg Cr)	63	890	1310

Elevated levels of ULTE4 have previously been reported in numerous conditions in which symptoms of MCA occur clinically, and for some of these disorders, cells other than MCs, notably eosinophils, are the primary source of cys-LTs.[11,12]

There are many reports of elevated leukotriene levels during anaphylaxis. Exercise, not related to food ingestion, has been confirmed as an anaphylactic trigger with documented hyperleukotrieneuria.[103] A 5.5- to 52-fold maximum increase in $ULTE_4$ excretion has been reported in anaphylaxis triggered by insect stings, exercise, or medications.[30] Another study of anaphylaxis found significantly increased levels of $ULTE_4$ (and 9α,11β-PGF_2) following allergen-induced anaphylaxis, but no increase in eosinophil-derived neurotoxin, thereby suggesting that MCs and not eosinophils were the LT source. Interval samples of urine showed that $ULTE_4$ levels peaked during the 3- to 6-hour collection interval following anaphylaxis, whereas the urinary 9α,11β-PGF_2 level peaked in the 0- to 3-hour interval. There was a significant correlation between peak $ULTE_4$ and 9α,11β-PGF_2 levels obtained during anaphylaxis.[31] Table V illustrates the marked attack-associated elevation in the $ULTE_4$ level as well as simultaneous elevations in 2,3 dinor 11β-$PGF_2\alpha$ and tryptase levels in one of our patients with MCAS. Taken together, these reports suggest that measurements of $ULTE_4$ will be useful in supporting a diagnosis of MCAS. Among our own patients with MCAS, we have documented marked increases in $ULTE_4$ excretion during MCAS.

Documentation of elevated urinary leukotrienes in patients with SM has appeared in several clinical reports. In one report cys-LT excretion positively correlated with simultaneously measured N-MH excretion.[104] Another report of 21 patients with bone marrow biopsy–confirmed SM had statistically increased ULTE4 when compared with a control group of patients with various potential MC-related symptoms such as spells, pruritus, dermatographia, angioedema, food intolerance, and others.[105]

BIOMARKERS ON THE HORIZON

General considerations

Certain mediators associated with MCs have not been demonstrated to have a role in the diagnosis or treatment of SM or MCAS. These include IL-8, chondroitin sulfate, serotonin, chromogranin A, neuropeptides, corticotropin-releasing hormone, platelet-activating factor, chymase, stem cell factor, nerve growth factor, and vascular endothelial cell growth factor.[97] The presence of KIT-mutated MCs especially when accompanied by IgE-triggered allergy and hereditary alpha tryptasemia predisposes patients to severe MCA episodes. In patients with idiopathic anaphylaxis, 14% were found to have SM. Here, allele-specific quantitative PCR for the c-kit D816V mutation was helpful in identifying idiopathic anaphylaxis patients with SM, but not monoclonal MCAS.[106,107]

IL-6 and heparin, however, may warrant further consideration.

IL-6

IL-6 is a product of B cells, T cells, monocytes, fibroblasts, keratinocytes, endothelial cells, mesangial cells, adipocytes,[108] and MCs themselves, where it can act in an autocrine fashion.[109] IL-6 enhances IgE-dependent histamine release from human peripheral blood–derived cultured MCs.[110] In SM, plasma levels of IL-6 are increased.[111,112] Plasma levels of IL-6 significantly correlate with disease category, severity of bone marrow pathology, organomegaly, cell count, prothrombin time, partial thromboplastin time, neutrophil numbers,[113] and the presence of osteoporosis.[112] All patients with ISM whose course progressed already had elevated IL-6 levels at the time of diagnosis.[114] In cultures of both bone marrow mononuclear cells of patients with SM and human MC lines, the presence of this mutation was associated with high levels of IL-6 production. The presence of the *c-kit* D816V mutation is closely related to IL-6 dysregulation, which was found to involve activation of signal transducer and activator of transcription 5 and PI3K pathways downstream of Asp816Val and was mediated by both the janus kinase 2 and by the mitogen-activated protein kinase/ERK1/2 pathways.[115]

Heparin

It might be expected that, because of MCs' content of heparin, MC disorders would commonly be accompanied by hemorrhagic sequelae. Yet, bleeding is rarely reported as a presenting or complicating symptom in SM.[116,117] A French national survey reported only 14 patients with clotting disorders, the most severe of which occurred in patients with advanced SM.[118] However, reports of prolongation of the activated partial thromboplastin time during anaphylaxis following wasp stings[119-121] suggests a heparin effect in this setting. In addition, a recent study demonstrated that increased plasma heparin levels in patients with SM and MCAS occurred following nonpharmacologic obstruction of venous flow for 10 minutes, yielding a sensitivity of 59% in patients with MCAS and 47% in patients with SM.[91] These findings warrant further validation before firm conclusions are possible.

CONCLUSIONS

Quantitation of urinary levels of MC mediator metabolites is a simple, noninvasive, and accurate method of documenting MCA and in supporting diagnoses of SM and MCAS. More information is necessary to establish a minimum required increase in these mediators at times of MCAS episodes before including any of them as markers for MCAS. For SM, excretion of 11β-$PGF_2\alpha$ 350% above the upper limit of normal and of N-MH 100% above the normal range correlated strongly with bone marrow findings of SM. Urinary levels of the metabolites of histamine, PGD_2, and LTC_4 can serve not only as biomarkers for these disorders but, importantly, have a pathophysiological role in causing symptoms. They provide valuable sources of information into the MC pathways triggered in individual patients and allow an effective targeted approach to treatment.

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