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REVIEW

# A concise, practical guide to diagnostic assessment for mast cell activation disease

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## Abstract

As recognition of mast cell (MC) involvement in a range of chronic inflammatory disorders has increased, diagnosticians' suspicions of MC activation disease (MCAD) in their chronically mysteriously inflamed patients have similarly increased. It is now understood that the various forms of systemic mastocytosis - diseases of inappropriate activation and proliferation of MCs seemingly driven by a small set of rare, usually constitutively activating mutations in assorted MC regulatory elements - comprise merely the tip of the MCAD iceberg, whereas the far larger and far more clinically heterogeneous (and thus more difficult to recognize) bulk of the iceberg consists of assorted forms of MC activation syndrome (MCAS) which manifest little to no abnormal MC proliferation and may originate from a far more heterogeneous set of MC mutations. It is reasonable to suspect MCAD when symptoms and signs of MC activation are present and no other diagnosis better accounting for the full range of findings is present. Initial laboratory assessment should include not only routine blood counts and serum chemistries but also a serum total tryptase level, which helps direct further evaluation for mastocytosis *vs* MCAS. Appropriate tissue examinations are needed to diagnose mastocytosis, while elevated levels of relatively specific mast cell mediators are sought to support diagnosis of MCAS. Whether assessing for mastocytosis or MCAS, testing is fraught with potential pitfalls which can easily yield false negatives leading to erroneous rejection of diagnostic consideration of MCAD in spite of a clinical history highly consistent with MCAD. Efforts at accurate diagnosis of MCAD are worthwhile, as many patients then respond well to appropriately directed therapeutic efforts.



**Key words:** Mast cell activation disease; Mastocytosis; Mast cell activation syndrome; Mast cell mediators; Tryptase; KIT mutations

**Core tip:** Mast cell activation disease (MCAD) is characterized by accumulation of genetically altered mast cells and/or abnormal release of these cells' mediators, affecting functions in potentially every organ system, often without causing abnormalities in routine laboratory or radiologic testing. Recent data suggest a high prevalence of MCAD. Thus, MCAD should be considered routinely in the differential diagnosis of patients with chronic multisystem polymorbidity of a generally inflammatory theme or patients in whom established diagnoses do not well account for the patient's presentation of symptoms consistent with mast cell mediator release. Mediator testing can be challenging but typically is manageable. Diagnostic efforts are worthwhile, as diagnosis often leads to effective therapy.

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## INTRODUCTION

Of hematopoietic origin, mast cells (MCs) are found in all human tissues, especially at the environmental interfaces and perivascular/perineural sites[1]. They serve largely as sentinels of environmental change and bodily insults and respond by releasing large and variable assortments of molecular mediators which directly and indirectly influence behavior in other local and distant cells and tissues to respond to changes and insults so as to maintain, or restore, homeostasis.

MCs and the related rare cutaneous disease urticaria pigmentosa (UP) were first discovered in the latter half of the 19$^{th}$ century[2,3], and the existence of seemingly even rarer systemic MC disease first became apparent in the middle of the 20$^{th}$ century[4,5]. For several decades it was thought that virtually all MC diseases were neoplastic, with symptoms resulting principally from accompanying inappropriate mediator release. Nearly a quarter century ago, the recurring somatic D816V mutation was discovered[6] in the dominant MC regulatory element, transmembrane tyrosine kinase receptor KIT[7], in a high proportion of patients with systemic mastocytosis (SM)[8,9]. This constitutively activating mutation has since been found to drive many features of SM including aberrant aggregation and spindled morphology of MCs in SM, tryptase and histamine overexpression, and aberrant MC surface co-expression of CD25[10-12]. Several other KIT mutations, also quite rare, have since been found in other cases of SM, mostly in KIT's kinase domain 1 at or near codon 816 or in the juxtamembrane region of KIT[11]. Recently, evidence has emerged that the KIT$^{D816V}$ mutation induces the above-mentioned immunohistochemical and morphological changes in affected MCs but seems not to be solely responsible for the clinical symptoms of the MC disease[13,14]. This finding fits well with previous reports in healthy people bearing the KIT$^{D816V}$ mutation in their peripheral blood leukocytes[15].

Around the same time as the discovery of KIT$^{D816V}$, though, the notion was first advanced that some portion of MC disease might be due to inappropriate mediator release with little to no accompanying MC proliferation[16]. This theory appeared validated when the first recognized cases of what is now called MC activation syndrome (MCAS) were published recently[17,18]. Although not yet independently confirmed, there soon followed provocative, repeated findings from one group of a very wide array of (presumably mostly constitutively activating) mutations scattered across all domains of KIT in small cohorts of MCAS patients[19,20]. Many of these patients appeared to bear multiple mutations in MC KIT, with no apparent recurring patterns. Similar mutational complexity has been found, too, across the spectrum of chronic myeloproliferative neoplasms (MPNs) within which the MC disorders reside[21], and in advanced mastocytosis itself[22].

In recognition of the fact that all MC disease is, first and foremost, disease of inappropriate MC activation, Akin *et al*[23] have proposed a new umbrella term of MC activation disease (MCAD) to describe the full spectrum of MC disease (Figure 1). MCAS is estimated to be more prevalent[13,24], but also more difficult to recognize, than other diseases traditionally ascribed to MC dysfunction. It also has been proposed that the various systemic MCAD variants and clinical phenotypes represent not distinct disease entities but rather varying presentations of a common generic root process of mast cell dysfunction[13]. The various forms of mastocytosis (principally cutaneous and systemic) may be the tip of a proverbial MCAD iceberg, fairly readily recognizable (in spite of their rarity) because of their defined unique immunohistochemical and relatively uniform clinical presentations, while the bulk of the iceberg - hidden below the waterline of easy clinical recognizability - may be a far larger, and far more heterogeneous, collection of variants of MCAS, some specifically named (*e.g.*, idiopathic anaphylaxis[18], cryopyrin-associated periodic syndrome[25]) but most not[23,26]. It seems logical that marked mutational heterogeneity would drive the marked heterogeneity of aberrant mediator expression and clinical presentation which are observed in MCAD and which can easily confound the diagnostician. For example, although MCAD can readily impact any or all systems in the body, cases have been described of MCAS causing hematologic presentations as diverse as pure red cell aplasia[27], erythrocytosis[28], and agranulocytosis[29].

Limited familial studies performed to date interestingly show high familial loading of MCAD despite a typical absence of germline mutations[24]; instead, most of the few families carefully studied show distinct sets of somatic mutations in the affected members with correspondingly varying clinical presentations. Clinical correlation suggests acquisition of the initial disease-causing mutations occurs relatively early in life, but additional mutations may develop in subclones over time. Efforts to reconcile the apparent dichotomy of high familial loading of MCAD with absence of apparent germline genetic mutations in affected families have led to thoughts that certain epigenetic alterations (GJM, unpublished data) may confer a state of genetic fragility which, upon interaction with varying biochemical milieus induced by assorted stressors (*e.g.*, infection), in turn


**Figure 1 Spectrum of mast cell disease.** SM-AHNMD: Systemic mastocytosis with associated clonal hematologic non-mast-cell-lineage disorder.

| Form of disease | | Features |
|---|---|---|
| Cutaneous mastocytosis | | Usually found in childhood; prevalence in the general population of approximately 0.01%[116,117]; various forms such as urticaria pigmentosa or telangiectasia macularis eruptiva perstans; diagnosed by skin biopsy |
| SM | | Usually emerges in middle age; prevalence in the general population of approximately 0.0003%[13] |
| | Indolent SM | Survival equal to that of the general population[118] |
| | Smoldering SM | Proposed category |
| | SM with associated clonal hematologic non-MC-lineage disorder | *e.g.*, SM with lymphoma or leukemia; outcomes are best if the SM and the AHNMD are treated concurrently[38] |
| | Aggressive SM | SM with end-organ failure such as hepatic fibrosis, portal hypertension, malabsorption, or cytopenias |
| | Mast cell leukemia | Rare; the most lethal and most therapeutically challenging of all mast cell diseases |
| Mast cell sarcoma | | Rare |
| Extracutaneous mastocytoma | | Rare |

**Figure 2 Diagnostic classification of mastocytosis[33].** SM: Systemic mastocytosis; AHNMD: Associated clonal hematologic non-mast-cell-lineage disorder.

induces MCAD-causing mutations in MC regulatory elements.

Diagnosticians willing to pursue the possibility of MCAD in their patients whose chronic multisystem polymorbidity (often, but not always, of a generally inflammatory theme) has defied extensive prior diagnostic efforts (typically focused on subspecialty-oriented symptom subsets rather than broad multisystem considerations) currently face many challenges including controversies in precise diagnostic criteria (particularly for MCAS), uncertainty regarding the utility of various diagnostic tests, and difficulties properly managing specimens for such testing. Whether assessing for mastocytosis or MCAS, testing is fraught with potential pitfalls which can easily yield erroneous conclusions that MCAD is not present and thereby potentially add yet much more time and cost to the very long and expensive path most patients with MCAD (particularly MCAS) require to establish diagnosis, let alone effective therapy. We describe here in detail our thoughts regarding current proposals for diagnostic criteria for MCAD and our approach to diagnostic testing for MCAD, *i.e.*, both mastocytosis and MCAS.

## DIAGNOSTIC CRITERIA FOR MCAD

Classification systems and diagnostic criteria for mastocytosis were first proposed in the 1980s[30,31]. Since 2001 the World Health Organization (WHO) consensus criteria have guided classification and diagnosis globally[32]. The latest revision of these criteria, published in 2008[33], divide mastocytosis broadly into cutaneous and systemic forms as well as the even rarer solid MC tumors of MC sarcoma and extracutaneous mastocytoma (Figure 2).

The WHO 2008 consensus diagnostic criteria for SM are shown in Figure 2. Given that it is now understood that MC KIT codon 816 mutations (a minor criterion) drive MC aggregation (a major criterion) and certain other minor criteria including MC spindling, tryptase overexpression, and CD25 co-expression, reorganization of the WHO diagnostic criteria for SM may be in order.

Recognition of the need for an alternative, MCAS-like diagnosis came about in part because of the discovery of patients whose clinical presentation was highly consistent with SM but who did not satisfy the major SM diagnostic criterion and only satisfied one or two of the minor criteria. In particular, many patients significantly affected by aberrant MC activation previously have been denied diagnosis of, and treatment for, MCAD because of a serum tryptase level < 20 ng/mL. Presently there are two principal proposals for diagnostic criteria for MCAS (Figure 3). Potential problems with the Valent *et al*[34] criteria include (1) non-recognition of many of the symptoms[28] that can result from MC activation; (2) lack of published validation that the described tryptase increase reliably distinguishes ordinary baseline fluctuation of tryptase from fluctuation induced by aberrant MC activation; (3) practical difficulties in providing/obtaining a specimen for serum total tryptase within 4 h of onset of an exacerbation of symptoms; and (4) practical difficulties in many patients in finding - in such a heterogeneous disease - MC-targeting agents that can effect at least partial response. Thus, for example, patients whose aberrant MC activation causes substantial muscle/joint/bone aching, constipation and abdominal pain, paresthesias, adenitis, and cognitive dysfunction but not the symptoms listed in these criteria would not qualify for the diagnosis. Similarly, a rise in serum tryptase (whose normal range typically is approximately 0.5 to 11 ng/mL) from 2.0 ng/mL to 4.2 ng/mL would be considered "20% + 2 ng/mL" evidence of aberrant MC activation, but a rise to 4.1 ng/mL would not. Furthermore, patients often are sufficiently disabled during a flare of symptoms that they cannot easily get to a medical center, and those who do travel to an urgent care facility or emergency department often encounter providers resistant to pursuing tests not needed for immediate care of the presenting symptoms.

WHO 2008 diagnostic criteria for systemic mastocytosis[33]

Major criterion:

Multifocal, dense aggregates of MCs (15 or more) in sections of bone marrow or other extracutaneous tissues and confirmed by tryptase immunohistochemistry or other special stains

Minor criteria:

1 Atypical or spindled appearance of at least 25% of the MCs in the diagnostic biopsy
2 Expression of CD2 and/or CD25 by MCs in marrow, blood, or extracutaneous organs
3 KIT codon 816 mutation in marrow, blood or extracutaneous organs
4 Persistent elevation of serum total tryptase > 20 ng/mL

Diagnosis of SM made by either (1) major criterion + any one or more minor criteria; or (2) any three minor criteria

Proposed diagnostic criteria for MCAS: Valent *et al*[34] criteria

1 Chronic/recurrent symptoms (flushing, pruritus, urticaria, angioedema, nasal congestion or pruritus, wheezing, throat swelling, headache, hypotension, and/or diarrhea) consistent with aberrant MC mediator release
2 Absence of any other known disorder that can better account for these symptoms
3 Increase in serum total tryptase of 20% above baseline plus 2 ng/mL during or within 4 h after a symptomatic period
4 Response of symptoms to histamine $H_1$ and/or $H_2$ receptor antagonists or other "MC-targeting" agents such as cromolyn

Proposed diagnostic criteria for MCAS: Molderings *et al*[26] criteria

Major criteria:

1 Multifocal MC aggregates as per WHO major criterion for SM
2 Clinical history consistent with chronic/recurrent aberrant MC mediator release (symptoms per Table 4 in[28])

Minor criteria:

1 Abnormal MC morphology as per WHO SM minor criterion 1
2 CD2 and/or CD25 expression as per WHO SM minor criterion 2
3 Detection of known constitutively activating mutations in MCs in blood, marrow, or extracutaneous organs
4 Elevation in serum tryptase or chromogranin A, plasma heparin or histamine, urinary N-methylhistamine, and/or other MC-specific mediators such as (but not limited to) relevant leukotrienes (B4, C4, D4, E4) or PGD2 or its metabolite 11-β-PGF2α

Diagnosis of MCAS made by either (1) both major criteria, or (2) the second major criterion plus any one of the minor criteria, or (3) any three minor criteria

**Figure 3 Diagnostic criteria for systemic mastocytosis and mast cell activation syndrome.** WHO: World Health Organization; MCs: Mast cells; MCAS: Mast cell activation syndrome; SM: Systemic mastocytosis PG: Prostaglandin.

Such resistance often persists even when the patient presents a prescription from a MC disease specialist specifically requesting MC mediator testing at times of such flares. Finally, in concert with the observed marked heterogeneity of the clinical presentation of MCAS (perhaps due to underlying marked mutational heterogeneity), some MCAS patients benefit little from the first few or several MC-targeting therapies tried, risking premature rejection of the diagnosis.

A potential problem with the Molderings *et al*[26] criteria for diagnosis of MCAS is the lack of mention of excluding other diagnoses (including mastocytosis) better accounting for the full range of findings in the patient, but such an exclusion would seem to be implicit. In practice, this scheme most often leads to diagnosis of MCAS by pairing of the second major criterion with the last of the minor criteria, and it appears to permit applying the diagnosis of MCAS - and therefore also pursuing therapy for MCAS - in a wider population of otherwise mysteriously chronically multi systemically ill patients, possibly increasing risk of misdiagnosis.

There have been no studies of the diagnostic accuracy or efficiency of these two diagnostic schemes. Also, at present there are no "gold standards" distinguishing levels of MC mediators seen in normal MC activation/reaction from levels seen in aberrant MC activation. We believe such distinctions will be difficult to develop, therefore also making it difficult to perform comparative studies of accuracy of different diagnostic schemes. Routine use of MC whole exome/genome sequencing and mutational analysis in patients with clinical histories suspicious for MCAD may become the most efficient route to definitively diagnosing MCAD, but currently the specific polymerase chain reaction (PCR) assay for $KIT^{D816V}$ (found often in SM but seldom in MCAS) is the only mutational analysis routinely available in most clinics. At present, though, it is worth noting that in view of the demonstrated great mutational complexity across the MPN spectrum, it may be premature to declare any given MCAS patient's disease as "non-clonal" based on negative clonality evaluations less complete than whole KIT sequencing. The term "uncertain clonality" may be more accurate.

Given the very limited mutational and clonality testing for MCAD presently available in most clinics, laboratory evaluation for MCAS will continue, at least for the near future, to depend far more on demonstration of elevated levels of MC-specific mediators.

## DIAGNOSIS OF MCAD

### *Establishing suspicion*

Accurate diagnosis of any condition begins with inclusion of the correct diagnosis in the considered differential diagnosis. Despite its rarity, development of clinical suspicion for mastocytosis is somewhat easier than for MCAS given the often flagrant nature of the clinical presentation, either with classic appearance of UP or telangiectasia macularis eruptiva perstans (TMEP) in cutaneous mastocytosis or with classic appearance of recurrent unprovoked flushing and/or anaphylaxis in systemic mastocytosis.

Initial suspicion of MCAS is more challenging due to its heterogeneity and, often, lack of flagrant acute presentation. Diagnosticians need to be cognizant that MCAD can affect every system, usually affects multiple systems, and usually manifests symptoms in a subacute or chronic waxing/waning or episodic fashion, though episodes also can arise acutely, *i.e.*, the so-called "flares" or "attacks" or "spells" that many patients have of one symptom set or another. Many symptoms often are categorized as inflammatory in nature (*e.g.*, pain, diarrhea), though non-inflammatory symptoms (or at least symptoms not traditionally thought to be inflammatory in nature) are prevalent, too (*e.g.*, fatigue, paresthesias).

Another clue that MCAD may be present is simply the oddity or unexpectedness of certain clinical events or findings in the patient. Given the very large array of mediators normally produced and released by the MC[35] and therefore potentially abnormally expressed in MCAD, with each mediator causing a unique, and usually wide, range of direct and indirect, local and distant effects, the potential for the disease to manifest "odd", "weird", "strange", "inexplicable" and "bizarre" clinical presentations is substantial. Such descriptors often are found throughout MCAD patients' charts for years prior to diagnosis. Especially when seen in the context of pre-existing chronic multisystem inflammatory illness, the appearance in the patient of "unusual" new clinical phenomena should provoke consideration of MCAD. (Of course, the diagnostician is abetted in such recognition by remaining abreast of the steadily enlarging scope of the chronic inflammatory diseases. For example, diabetes mellitus type 2, obesity, and atherosclerotic vascular disease have been recognized as chronic inflammatory diseases only relatively recently.)

By the time MCAD is diagnosed, most patients have seen many providers, have undergone extensive testing (often frustratingly yielding normal or non-specifically/minimally abnormal results), and have been assigned many diagnoses (often with less than a full measure of confidence) which explain assorted subsets of findings but do not well account for the full range of findings, including chronicity often dating back decades. Treatments for these preliminary diagnoses sometimes help somewhat but often do not help at all, further perplexing the diagnostician. It is reasonable to suspect MCAD when at least several symptoms and signs of MC activation are present (Table 1)[26,28,36,37] and no other diagnosis better accounting for the full range of findings is present. In particular, presence of a definitively diagnosed condition (*e.g.*, lymphoma) which does not well account for all symptoms and findings (*e.g.*, presyncope, erythrocytosis, *etc.*), or poor response of a definitively diagnosed condition to standard treatment for that condition, should raise suspicion for the presence of either a significant independent diagnosis or an alternative underlying ailment better accounting for the full range of symptoms and findings. In such cases, definitive diagnosis of a comorbid (and potentially even underlying) MCAD permits dual-directed therapy[38] which may lead to improved outcomes. Standardized detection of a MC mediator release syndrome can be achieved by a validated questionnaire (Figure 4). Although routine complete blood counts (CBCs) and metabolic panels often are confoundingly normal in MCAD patients, it is also the case that abnormalities in these and other common blood tests in these patients are commonly seen and are typically modest and stable. Frequent, though not necessarily constant, relative or absolute monocytosis, eosinophilia, basophilia, and/or reactive lymphocytosis, typically to just modest degrees, can be seen along with similar patterns of abnormality in routine chemistries such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP) (LBA and GJM, unpublished data), hyperbilirubinemia, and diet-independent hypercholesterolemia and hypertriglyceridemia[39]. Viewed in encounter-specific isolation, such abnormalities often raise no concern and garner no further attention, but trend analysis made easy by electronic medical record systems can quickly highlight the persistence of these abnormalities and permit expansion of the considered differential diagnosis to include entities such as MCAD which can account for these additional findings.

### *Mastocytosis or MCAS? Initial laboratory assessment*

It is now understood that the serum total tryptase level much more reflects the total body MC load than the total body MC activation state[40-42]. As such, serum tryptase is expected to be elevated in mastocytosis but usually is elevated little to none in MCAS. "Spillover" into blood circulation of tryptase and other MC mediators initially released in the tissues can be influenced by many factors. Hence, a normal value for a mediator in blood or other bodily fluid does not rule out its unregulated release in tissue. In fact, about 20% of SM patients present with serum tryptase < 20 ng/mL[37,43,44], but such patients rarely harbor the more advanced forms of SM [aggressive SM (ASM) and MC leukemia (MCL)] which require therapeutic approaches different from those used for the more common indolent SM and MCAS. Marrow aspiration/biopsy, an uncomfortable procedure for some patients, is warranted in cases of suspected mastocytosis but seldom yields diagnostic findings in cases of MCAS not already manifesting significant hematologic abnormalities (LBA and GJM, unpublished data). Therefore, a serum tryptase persistently elevated to > 20 ng/mL in two or more spec-

**Table 1 Symptoms and findings in mast cell activation disease**

| System | Potential manifestations of MCAD (most are chronic, low-grade; some are persistent, but many are either episodic or fluctuant) |
|---|---|
| Constitutional | Fatigue, malaise, asthenia, "chronic fatigue syndrome", subjective and/or objective hyperthermia and/or hypothermia, "sense of feeling cold much of the time", sweats/diaphoresis (not always nocturnal), flushing, plethora or pallor, increased or decreased appetite, early satiety, weight gain or loss, pruritus, chemical and/or physical environmental sensitivities (often odd) |
| Dermatologic/ integument | Rashes and lesions of many sorts (classic urticaria pigmentosa, "freckles", telangiectatic/angiomatous lesions, xerosis, warts, tags, folliculitis, ulcers, dyshidrotic eczema, diffusely migratory but sometimes focally persistent patchy macular erythema), pruritus (often diffusely migratory, sometimes aquagenic), flushing, angioedema, striae, dermatographism, hair thinning and alopecia, onychodystrophy (brittle nails, longitudinal ridges), poor healing |
| Ophthalmologic | Irritated eyes, increased or decreased lacrimation, suffusion, conjunctivitis, episodic difficulty focusing, lid tremor/tic (blepharospasm), solar sensitivity, infectious or sterile inflammation |
| Otologic/osmic | Infectious or sterile otitis externa and/or media, hearing loss or hyperacusis, tinnitus, otosclerosis, dysosmia, coryza, congestion |
| Oral/oropharyngeal | Pain or irritation (sometimes "burning"), leukoplakia, fibrosis, lichen planus, ulcers, sores, angioedema, dental decay, dysgeusia, throat tickle/discomfort/irritation/pain, post-nasal drip |
| Lymphatic | Adenopathy, usually sub-pathologic and often waxing/waning in size, sometimes asymptomatic but not uncommonly tender, sometimes focal, sometimes migratory, pathology usually shows reactive lymphocytosis or sometimes an atypical non-specific lymphoproliferative disorder; left upper quadrant discomfort (likely from release of mediators from splenic mast cells with or without detectable splenomegaly) |
| Pulmonary | Rhinitis, sinusitis, pharyngitis, laryngitis, bronchitis, pneumonitis (often confused with infectious pneumonia), cough, dyspnea (often low-grade, inconstant, "I just can't catch a deep breath" despite normal pulmonary function tests), wheezing, obstructive sleep apnea, pulmonary hypertension |
| Cardiovascular | Presyncope (lightheadedness, weakness, dizziness, vertigo) and/or syncope (patients may have been diagnosed with postural orthostatic tachycardia syndrome or neurocardiogenic syncope), hypertension and/or hypotension, palpitations, dysrhythmias, chest discomfort or pain (usually non-anginal in character), coronary and peripheral arterial atherosclerosis/spasm/infarction, idiopathic acute or chronic heart failure (*e.g.*, takotsubo), aneurysms, hemorrhoids, varicosities, aberrant angiogenesis (hemangiomas, arteriovenous malformations, telangiectasias), migratory edema (often non-dependent and with normal cardiac and renal function) |
| Gastrointestinal | Aerophagia, angioedema in any segment of the luminal tract, dysphagia (often proximal, possibly due to pharyngeal angioedema), bloating/gas, pain/inflammation (often migratory) in one or more segments of the luminal tract (from esophagitis to proctitis) and/or one or more solid organs (*e.g.*, hepatitis, pancreatitis), queasiness, nausea, vomiting (sometimes "cyclical"), diarrhea and/or constipation (often alternating), malabsorption (more often selective micronutrient malabsorption than general protein-calorie malabsorption), ascites either from portal hypertension and/or peritoneal serositis; gastroesophageal reflux disease (often "treatment-refractory") and inflammatory/irritable bowel syndrome are common pre-existing diagnoses |
| Genitourinary | Inflammation (often migratory) in one or more segments of the luminal tracts (ureteritis, cystitis, urethritis, vaginitis, vestibulitis) and/or one or more solid organs (*e.g.*, nephritis, prostatitis), chronic kidney disease, endometriosis, chronic low back pain or flank pain or abdominal pain, hydronephrosis (likely from ureteral angioedema), infertility, erectile dysfunction, decreased libido; in the appropriate setting of multisystem morbidity, miscarriages should prompt consideration of antiphospholipid antibody syndrome potentially due to MCAD |
| Musculoskeletal | Clinical myositis, often diffusely migratory (fibromyalgia is a common pre-existing diagnosis), subclinical myositis (*i.e.*, asymptomatic elevated creatine kinase not otherwise explained), arthritis (typically migratory), joint laxity/hypermobility (patients may have been diagnosed with Ehlers-Danlos Syndrome Type Ⅲ), osteoporosis/osteopenia, osteosclerosis, sometimes mixed osteoporosis/osteopenia/osteosclerosis; MCAD-driven musculoskeletal pain not uncommonly is poorly responsive to non-steroidal anti-inflammatory drugs and narcotics |
| Neurologic | Headache (esp. migraine), presyncope and/or syncope, peripheral (usually distal) sensory and/or motor neuropathies including paresthesias, tics, tremors (typically resting), chronic inflammatory demyelinating polyneuropathy, seizure disorders (can be "treatment-refractory"), pseudoseizures, dysautonomia |
| Psychiatric | Mood disturbances (*e.g.*, anger, depression), bipolar affective disorder, attention deficit-hyperactivity disorder, post-traumatic stress disorder, anxiety and panic, psychoses, memory difficulties, word-finding difficulties, other cognitive dysfunction, wide variety of sleep disruptions |
| Endocrinologic/metabolic | Abnormal electrolytes (including magnesium) and liver function tests, delayed puberty, dysmenorrhea, endometriosis, osteosclerosis and/or osteoporosis, hypothyroidism, hyperthyroidism, dyslipidemia, hyperferritinemia, selective vitamin and/or other micronutrient deficiencies, weight change, possibly diabetes mellitus |
| Hematologic/coagulopathic | Polycythemia or anemia (may be macrocytic, normocytic, or microcytic), leukocytosis or leukopenia, chronic (usually mild) monocytosis or eosinophilia or basophilia, thrombocytosis or thrombocytopenia, arterial and/or venous thromboembolic disease, "easy" bruising/bleeding; in mast cell activation syndrome the marrow usually does not show increased (or even flow-cytometrically aberrant) mast cells; marrow histology often read as normal or as unspecified myelodysplastic/myeloproliferative syndrome; standard cytogenetic studies are almost always normal or show culture failure |
| Immunologic | Type Ⅰ, Ⅱ, Ⅲ and Ⅳ hypersensitivity reactions, increased risk for malignancy, autoimmunity, impaired healing, increased susceptibility to infection, elevated or decreased levels of one or more isotypes of immunoglobulin; modest monoclonal gammopathy of undetermined significance not uncommon |

MCAD: Mast cell activation disease.

imens facilitates an initial decision whether to evaluate further for SM with biopsies as appropriate including at least marrow, and possibly also skin, upper and lower gastrointestinal (GI) tract mucosa, and potentially other tissues, especially those at the environmental interfaces. An initial serum tryptase < 20 ng/mL makes SM (especially ASM and MCL) much less likely, and since the prognosis of, and therapeutic approach toward, indolent SM and MCAS are presently indistinguishable, there appear to be no adverse consequences if the clinician initially misdiag-

*Clinical signs*

The patient complains about recurring or continuing burning and/or crampy abdominal pain of unknown cause and/or recurring or continuing diarrhea of unknown cause and/or frequently intense meteorism/gassiness (independent of the composition of diet) and/or about episodically occurring nausea. □ 1

The symptoms respond to treatment with H1-antihistamines. □ 1

The progression of the symptoms occurred in episodes □ 1
with symptom-free periods becoming shorter. □ 1

The patient complains about episodically occurring burning and/or choking chest pain attacks, which are often experienced as life-threatening. Electro cardiographic findings are without pathological signs. □ 1

The patient complains about occasional or continuing pain in the urinary bladder and/or pelvis accompanied by painful desire to void and/or blood in the urine. There is no bacteriuria. □ 1

The patient complains about occasional or continuing paresthesia (burning, pins and needles, numbness) and/or pain which does not respond to treatment with analgesics. □ 1

Gastroscopy and biopsies from the stomach and duodenum
are without pathological findings. □ 0
or
show minor signs of inflammation. □ 1
or
show *Helicobacter pylori*- and NSAID-negative erosion and/or ulcer. □ 3
or
show clusters of mast cells and/or a considerable number of spindle-shaped mast cells and/or CD25-positive mast cells. □10

Colonoscopy and intestinal biopsies
are without pathological findings. □ 0
or
show minor signs of inflammation. □ 1
or
show melanosis coli (abuse of anthra-cenediones ruled out). □ 1
or
show clusters of mast cells and/or a considerable number of spindle-shaped mast cells and/or CD25-positive mast cells. □10

The patient reports the following signs of episodically occurring symptoms of autonomic dysfunction:
tachycardia or
palpitation/dysrhythmia □ 1
flush (redness, feeling of heat) □ 2
hot flash, sweat □ 2
paroxysmal hypo/hypertension with
dizziness to the point of syncope □ 2

Although there are no pathological findings in routine laboratory parameters and imaging methods, the patient present with
a pronounced asthenia. □ 1
fatigue. □ 1
loss in weight. □ 1

During the symptomatic periods of the disorder the patient is
afflicted with anal pruritus and/or anal eczema. □ 1
Intestinal adhesions are present without prior history of abdominal surgery □ 1

*Triggering factors*

Deprivation of sleep □ 1
Fasting for 24 h □ 1
Histamine containing food (*e.g.*, red wine, cheese, tuna) □ 1

*Laboratory parameters*

The patient shows signs of a bleeding diathesis (*e.g.*, abnormal secondary bleeding or bruises after minimal trauma and/or lesions). □ 1
During the symptomatic periods of the disorder the patient showed, at least once, hyperbilirubinemia (up to 2.5 mg/dL), and/or an increase of trans amidases (up to twice their upper limits of normal) and/or diet-independent hypercholesterolemia (up to 300 mg/dL). □ 1

There are low titers of autoantibodies without clinical signs in the organs or tissues against which the autoantibodies are directed. □ 1

The serum total tryptase was normal. □ 0
or
was elevated > 11 and < 20 ng/mL. □ 3
or
was elevated more than 20 ng/mL. □10

The level of heparin in blood was normal. □ 0

| | |
|---|---|
| or | |
| was elevated > 0.05 anti-Factor Xa units/mL. | □ 3 |
| The level of N-methylhistamine in a 12-hurine collection was normal. | □ 0 |
| or | |
| was marginally elevated. | □ 1 |
| or | |
| was elevated up to tenfold of the reference value. | □ 5 |
| or | |
| was elevated by more than tenfold of the reference value. | □10 |
| *Imaging methods* | |
| The patient has splenomegaly and/or hepatomegaly. | □ 1 |
| The patient has bone pain with signs of osteoporosis and/or osteopenia and/or osteosclerosis. | □ 1 |
| *Medical history* | |
| The patient shows involvement of the skin in terms of | |
| brown-Reddish maculopapulous rash/eruption. | □ 2 |
| angioedema of the lips, lids of the eye, infraorbital. | □ 2 |
| pruritus (itching) without rash/eruption and/or disease-related folliculitis | □ 1 |
| a clear increase in the number of telangiectasias. | □ 1 |
| The patient reports sudden attacks of migraine-like headache. | □ 1 |
| The patient reports memory loss (ability to remember names or words) and/or concentration difficulty and/or sleep disturbances. | □ 1 |
| The patient reports tinnitus attacks and/or ocular discomfort (dry eyes, red eyes, stinging eyes) and/or rhinorrhea/chronic nasal congestion and/or stomatitis (score if two or more of these symptoms are present). | □ 1 |
| The patient reports non-allergic respiratory ailments such as asthma, compulsion to clear the throat, titillating/ticklish feeling in the respiratory tract and/or shortness of breath during routine tasks. | □ 1 |
| In the past, common viral infections of the upper respiratory tract were frequently complicated by bacterial superinfection. | □ 1 |
| The patient can state precisely the date of the first clinical manifestation of the mast cell mediator release syndrome because it appears to him to be associated with an infectious disease. | □ 1 |

**Figure 4 Validated questionnaire to recognize symptoms as part of a mast cell activation disease in a standardized manner (modified from[39,119]).** The indicated values for those items acknowledged by or found in the patient are summed. A total score above 8 but less than 14 indicates a pathological activation of mast cells. At a total score of 14 and more, a systemic mast cell mediator release syndrome is clinically verified. NSAID: Non-steroidal anti-inflammatory drugs.

noses the uncommon low-tryptase SM as MCAS.

Tryptase has been well established as a highly specific MC mediator[45]. Its biology is complex; many isoforms with different behaviors and functions have been elucidated[46]. It is heat-labile[47,48] and has a relatively short half-life *in vivo* (from 6-8 min in healthy subjects to 1.5-2.3 h in patients with hypersensitivity reactions), longer (approximately 4 d) in separated serum[34,49]. The WHO 2008 diagnostic criteria for mastocytosis call only for the measurement of total serum tryptase, setting a threshold of 20 ng/mL as the minimum level consistent with a diagnosis of SM[33]. It is unclear whether measurement of specific isoforms of tryptase would be beneficial in diagnosing MCAD in any form, and thus at present only the measurement of total serum tryptase can be recommended in the evaluation of a patient suspected of having MCAD. Although the serum total tryptase usually is not elevated in MCAS[20,26], an elevated level (though almost always < 20 ng/mL) can be found in a minority of MCAS patients, and any elevation at all can help buttress a diagnostician's suspicions of the involvement of MC activation in the patient's illness.

If the serum tryptase persistently exceeds 20 ng/mL, or if other clinical features of the presentation are characteristic for SM (*e.g.*, initial onset of symptoms in middle age, MCs observed in the peripheral blood smear, *etc.*), then further evaluation for SM is warranted. Marrow aspiration/biopsy is a standard part of such evaluation, and given both the patchy distribution of the disease in involved tissues and the observation that unilateral marrow biopsies are non-diagnostic in one-sixth of patients ultimately diagnosed with SM[50], bilateral aspirations/biopsies are preferred. Core biopsies should undergo routine staining as well as MC-targeted immuno/histochemical staining (*e.g.*, CD117, CD25, CD2, tryptase, Giemsa, toluidine blue, Alcian blue, *etc.*). There is no particular subset of these stains which has been established as a standard initial assessment. It has been proposed that CD30, too, be routinely examined when assessing for MCAD[38]. Also, CD68 - more classically associated with macrophages - can be displayed by MCs and thus may be useful in diagnosing MCAD[51]. All of the above having been said, MCs express surface CD117 (the extracellular portion of *c-kit*) roughly an order of magnitude more brightly than any other CD117-expressing cell, so often only CD117 staining is needed to estimate MC density and to characterize aggregation. Each aspirate should be sent for standard cytogenetic

analysis as well as multi-color flow cytometric assessment for co-expression of cell-surface doublets CD117/CD25 and CD117/CD2; occasionally even the triplet of CD117/CD25/CD2 is seen[52]. These flow cytometric signatures are widely considered to be pathognomonic for monoclonal MC disease. Each aspirate should be subjected to the full extent of MC mutational analysis available at the time, though at present this typically is limited to PCR analysis for the $KIT^{D816V}$ mutation. If leukocytosis, erythrocytosis, or thrombocytosis is present, assessment is also warranted for the Janus kinase 2 (JAK2) mutations often found in the MPNs (and perhaps the $MPL^{W515L/K}$ mutation, too, in cases of leukocytosis or thrombocytosis without erythrocytosis which might signal the presence of essential thrombocythemia or myelofibrosis). Similarly, if prominent eosinophilia is observed, tyrosine kinases platelet-derived growth factor receptor alpha (PDGFRα), PDGFRβ, and fibroblast growth factor receptor 1 should be assessed for mutations as recently reviewed elsewhere[53].

The presence of skin lesions characteristic of UP or TMEP warrants biopsy of one or more such lesions, to be immediately processed with routine staining and the above-noted immunohistochemical staining plus assessment for clonality with flow cytometry. As solid tissue flow cytometry requires fresh tissue but PCR mutation analysis does not, it may be reasonable to defer PCR of skin lesion biopsies until other testing finds at least some evidence suggestive of MC disease.

Spontaneously appearing/disappearing, diffusely migratory macular erythematous patchy rashes are common in MCAS, as are scatterings of small ulcerative lesions, too. In the authors' experience, though, biopsies of such sites rarely reveal increased or otherwise aberrant MC populations, suggesting such lesions may be merely the end result of aberrant release of mediators from distantly located MCs.

If clinical suspicion for SM persists (whether due to serum tryptase persistently > 20 ng/mL or other factors) but two or more marrow biopsies (and possibly also biopsies of suspect skin lesions) are negative, upper and lower endoscopic examination of the gastrointestinal tract is reasonable. Biopsies should be performed of any macroscopically apparent lesions, but blind biopsies of macroscopically normal mucosa should be pursued, too, at multiple points along the tract. All such biopsies should be processed as described above for skin biopsies. Several studies have reported that mast cell counts in GI or genitourinary (GU) tract mucosal biopsies normally number fewer than 20 per high power field[54-58]. However, cut-offs between "normal" and "abnormal" numbers of non-aggregated mast cells are not addressed in either of the two currently proposed diagnostic schemes for MCAS. Furthermore, it is important to remember that the diagnosis of any form of MCAD rests in meeting a set of criteria, so an isolated finding of "increased" tissue mast cells does not establish the diagnosis regardless of the cut-off used to define "increased", and especially when such cells are not tightly clustered. In the context of other data meeting diagnostic criteria, though, a finding of "increased" tissue mast cells lends additional credence to the diagnosis.

MCs are highly pleomorphic. On routine hematoxylin and eosin (H and E) staining, MCs can be indistinguishable from lymphocytes, plasma cells, macrophages, histiocytes, or spindle cells[59,60]. Thus, in MC disease, not all "lymphocytes" seen with H and E staining are necessarily lymphocytes, and immuno/histochemical staining is required in cases of suspected MCAD. Not infrequently, because of their chronic idiopathic (and often treatment-refractory) gastrointestinal symptoms, patients being evaluated for MCAD have previously undergone GI tract endoscopies with biopsies. Thus, simple reassessment of archived tissue with MC-targeted immuno/histochemical staining may be sufficient to demonstrate that the "benign mucosa" or "mild chronic inflammation" previously seen with H and E staining actually harbors increased MCs. As PCR testing can be performed on preserved tissue, positive immuno/histochemical findings on archived tissue should lead, if sufficient tissue remains, to mutation analysis as possible.

Clinical judgment is required as to whether respiratory or GU tract biopsies are warranted in particularly diagnostically challenging cases, but if pursued, such tissues should be processed as for skin and GI tract biopsies.

Newly or persistently pathologically enlarged (> 1 cm) lymph nodes require biopsy to rule out malignant lymphoproliferative and granulomatous disorders (regardless of whether such disorders can be proven to be independent of, or consequent to, MCAD). However, diffusely migratory, spontaneously waxing/waning adenopathy and/or adenitis is common in MCAD and far more often is a reactive rather than malignant process, though the reactive patterns seen on biopsy can be bizarre (*e.g.*, sinus histiocytosis) and can have the pathologist on the verge of declaring a malignancy. In patients diagnosed with MCAD (or being evaluated for suspected MCAD) who have a longstanding history of spontaneous enlargement of nodes to pathologic size soon followed by spontaneous regression of such adenopathy, the new appearance of a modestly pathologically enlarged lymph node can be watched closely without reflexively proceeding to biopsy, but persistence or progression of adenopathy beyond the range, or outside of the pattern, the patient has usually experienced may warrant biopsy. Node biopsies should be excisional whenever possible and should be submitted not only for the above-noted analyses for MC disease but also for routine analyses for lymphoproliferative disorders and perhaps even - in appropriate clinical settings - specific plasma cell dyscrasias or granulomatous diseases such as amyloidosis, Castleman's disease, or sarcoidosis.

In addition to checking a serum tryptase, other initial laboratory assessment for MCAD (whether suspected to be mastocytosis or MCAS) should include a variety of common tests as listed in Figure 5. As MCAD commonly causes osteolysis (and, less commonly, osteosclerosis - and occasionally even both processes at different

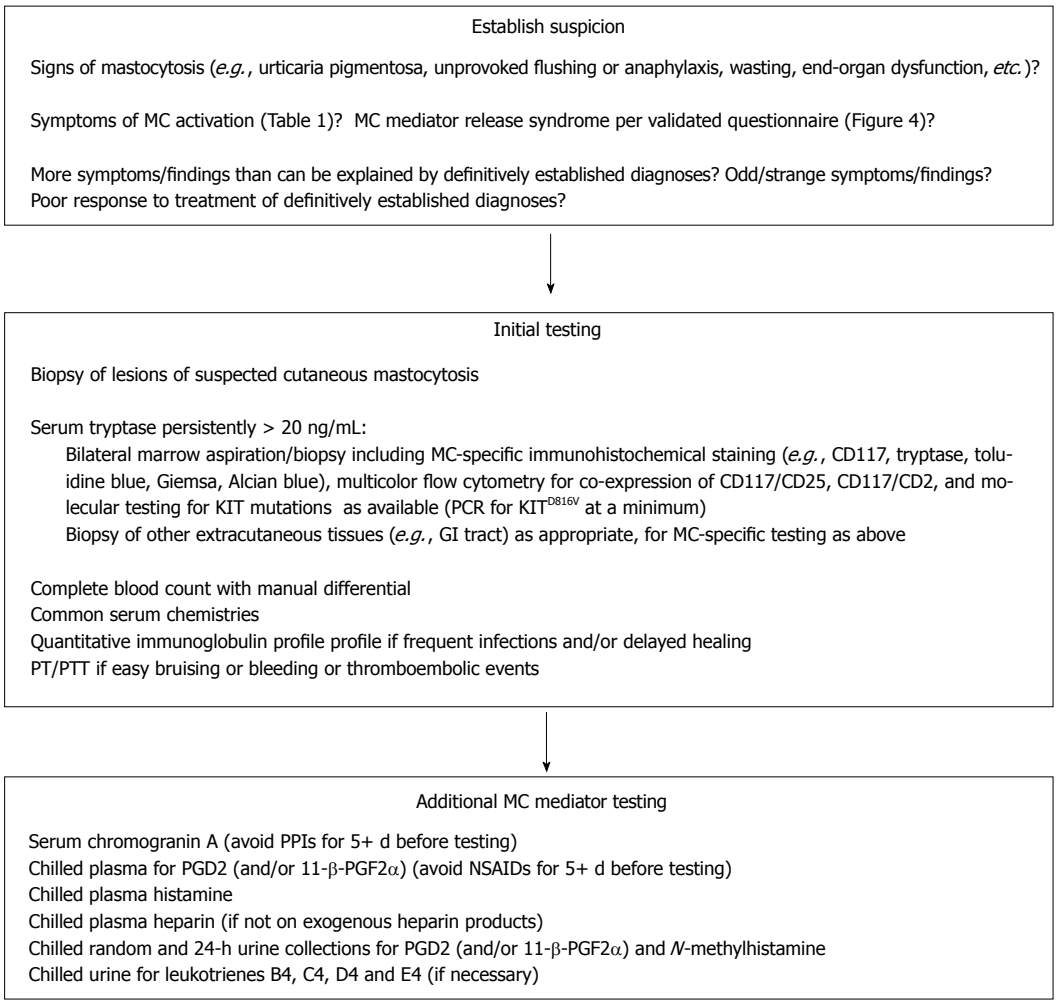


**Figure 5 Diagnostic approach to mast cell activation disease.** MCs: Mast cells; MCAS: Mast cell activation syndrome; GI: Gastrointestinal; PG: Prostaglandin; PCR: Polymerase chain reaction; NSAID: Non-steroidal anti-inflammatory drugs.

sites in the same patient), it is appropriate to establish a baseline for bone densitometry after the diagnosis of MCAD has been established, as the finding of osteopenia or osteoporosis has therapeutic implications. MCAD can drive erythropenia or erythrocytosis (either of which may be macrocytic, normocytic, or microcytic, engendering appropriate differential diagnostic considerations of secondary processes), leukopenia or leukocytosis, and/or thrombocytopenia or thrombocytosis, but there are no findings in the CBC that are specific for MCAD (other than significant numbers of circulating mast cells signaling mast cell leukemia). Also, as previously noted, certain subtle abnormalities are not uncommonly seen in the leukocyte differential, but often the differential is as normal as the CBC. Indeed, it is a confounding point for hematologists that this disease of fundamentally hematologic classification often presents with not a single abnormality in the CBC or differential. However, many MCAD patients - with either mastocytosis or MCAS - do manifest subtle to gross abnormalities in one or more elements of the CBC, providing the diagnostician important opportunities to consider alternative diagnoses. Of note, though, gross abnormalities in the CBC in advanced mastocytosis may be due more to marrow replacement by MCs, while gross abnormalities in the CBC in less advanced mastocytosis and MCAS are likely due more to MC mediator-driven interference with normal hematopoiesis.

Many MCAD patients also manifest abnormalities in the common serum chemistries, again typically modest in degree but providing the diagnostician opportunities to consider alternative diagnoses regardless of whether they may have risen independently of, or consequent to, MCAD.

Both hypergammaglobulinemia (usually a polyclonal inflammatory phenomenon but occasionally a monoclonal gammopathy of undetermined significance or more advanced clonal plasma cell dyscrasia) and hypogammaglobulinemia can be seen in MCAD[61]; patients sometimes have been previously diagnosed with common variable immunodeficiency. Markedly IgG-deficient patients (< 400 mg/dL) who have suffered major infections or substantial healing complications may be candidates for prophylactic immune globulin therapy.

Histories of "easy" bruising and/or bleeding are frequent amongst MCAD patients. Multiple mechanisms

are possible (and not necessarily mutually exclusive) including coagulation factor deficiencies, antiphospholipid antibodies, and/or MC release into the local tissues of (antithrombin-activating) heparin. However, fibrinolysis (as stimulated by heparin release[62] and other fibrinolytic mediators such as tissue plasminogen activator[63]) appears likely to be the dominant cause, explaining why antifibrinolytics such as tranexamic acid can be helpful in controlling MCAD-driven bleeding. The prothrombin time (PT) and activated partial thromboplastin time (aPTT) remain reasonable screening tests in patients with suspected MCAD who report histories of easy bruising/bleeding, but the diagnostician should be aware that local MC release of mediators with sufficient direct or indirect anticoagulant effect to cause local bleeding usually do not provoke systemic anticoagulant effect sufficient to cause rises in PT or aPTT to abnormal levels. Thus, measurement of fibrinolytic mediators[63], as proximate to the onset of bleeding as possible, may be required to prove fibrinolysis if felt clinically important. Otherwise inexplicable prolongations in PT or aPTT should prompt appropriate further evaluation for clotting factor deficiencies. Also, assessment for antiphospholipid antibodies may not be unreasonable in suspected MCAD patients with otherwise inexplicable abnormalities (high or low) in either PT or aPTT and histories of not only easy bruising/bleeding but also thromboembolism or miscarriage.

If not already performed, a careful evaluation for known hypercoagulable disorders should be performed in the patient with suspected MCAD and a history of idiopathic thromboembolism. Such evaluation should include, among other considerations, determination of the plasma level of Factor Ⅷ (produced by various sources including the MC[64] and associated with hypercoagulability[65]) and, with a history of portal/splanchnic vein thrombosis, assessment for the JAK2$^{V617F}$ mutation which might suggest the occult presence of an MPN[66]. Testing for inborn hypercoagulable disorders may not be warranted in cases of initial thromboembolism at ages above 40.

Most patients presenting for evaluation of MCAD have been chronically unwell and have undergone extensive diagnostic evaluation, but the diagnostician considering MCAD nevertheless must carefully review past evaluations and ensure other diagnostic considerations potentially fitting the patient's unique course have been excluded (*e.g.*, hypothyroidism, celiac sprue, Epstein-Barr virus disease, carcinoid, amyloidosis, porphyria, sarcoidosis, *etc.*). Even if the root cause of the patient's multisystem unwellness is MCAD, identification of specific secondary phenomena (*e.g.*, autoimmunity, infection, malignancy, *etc.*) may permit better outcomes via dual-directed therapy.

### Laboratory assessment of other MC-specific mediators

If the serum tryptase is < 20 ng/mL and there are no other clinical hints that SM is more likely than MCAS, then an initial laboratory survey of other MC mediators should be pursued in patients clinically suspected of having MCAS. In the authors' opinion, such an initial mediator survey for evidence of MCAS should include serum chromogranin A; chilled plasma for prostaglandin $D_2$ ($PGD_2$) (and/or 11-β-$PGF_{2\alpha}$); chilled plasma histamine; chilled plasma heparin (in patients not on exogenous heparin products); and chilled random and 24-h urine collections for $PGD_2$ (and/or 11-β-$PGF_{2\alpha}$) and *N*-methylhistamine[26]. In some situations (see below), urinary levels of leukotrienes B4, C4, D4 and E4 may also be worth pursuing[26,67].

Although specimen collection as soon as possible following an acute flare of symptoms is ideal, there is no need to wait for such an event when initially assessing the patient with longstanding baseline symptoms consistent with aberrant MC mediator release. However, if the initial laboratory assessment in a patient with a history suspicious for MCAD is negative, repeat testing is usually warranted but preferably should be deferred until the presentation of an acute flare. If possible, hourly determinations of serum tryptase, plasma $PGD_2$ and histamine, and spot urinary $PGD_2$ and *N*-methylhistamine should be pursued at baseline and over the next 2-3 h as a flare evolves.

Chromogranin A (CgA) is another known MC product[35,68] which appears quite heat-stable[69] and was found elevated in the serum in 17% (21 out of 122) of a cohort of MCAD patients investigated (LBA, unpublished data). Elevated serum levels of CgA can be helpful toward diagnosing MCAS, but other potential causes of such elevations (heart failure, renal insufficiency, proton pump inhibitor (PPI) use, and neuroendocrine cancer) should be excluded. Changes in serum CgA level in response to initiation or cessation of PPI therapy are fairly reliable and rapid (within 5 d[70]) but highly variable in magnitude[71,72], likely due to multiple mechanisms including pharmacogenomic polymorphisms (largely in cytochrome p450 isoenzyme 2C19[73]) and increase in density of CgA-secreting enterochromaffin cells (especially in gastric tissue) in response to gastric acid reduction[74]. Cessation of PPI therapy at least 5 days prior to submission of a serum sample for CgA assessment should permit an accurate gauge of the baseline CgA level.

Although it was the first MC mediator to be discovered[75,76] and is a highly sensitive and specific indicator of MC activity[16,63], the plasma heparin level is difficult to measure accurately in the clinical setting. The metabolism of heparin is complex[77], but it is clear that it has a short half-life (generally less than one hour) and decomposes rapidly even at chilled temperatures[63]. Seidel *et al*[63] reported an upper limit of normal plasma heparin of 0.02 anti-Factor Xa units/mL, but the utility of assessing the plasma heparin level for evidence of MCAS may be constrained by the fact that most of the commercially available assays have a lower limit of detectability of 0.05 or 0.10 anti-Factor Xa units/mL since such assays are used far more to assess anticoagulant adequacy of therapeutic heparinization than to assess endogenous heparin levels. However, about 50% of MCAD patients

will manifest a plasma heparin level exceeding 0.05 anti-Factor Xa units/mL on commercially available assays (105 out of 202 MCAD patients in a current study; unpublished data), and in such patients whose detectable plasma heparin levels cannot be attributed to therapeutic heparin or to the only other known endogenous source of heparin in humans (basophils), MC activation almost certainly is the source.

Like heparin, histamine is slightly less specific for MC disease than tryptase in that histamine is also released by basophils. Unlike heparin, histamine is not particularly heat-labile[78] but nevertheless has a short half-life *in vivo*[79,80] (estimates have ranged from 1 min to 1 h and may vary based on increased histaminase activity during anaphylaxis[81]). Histamine appears to be stable in separated plasma at room temperature for at least 48 h[82]. However, because histamine assays often must be performed at distant reference laboratories (requiring specimen storage and transport exceeding 48 h), plasma specimens for histamine levels should be kept continuously chilled at all stages of handling. Histamine's major metabolite, *N*-methylhistamine, has a longer half-life *in vivo*[80] and may be a preferable measure over histamine, especially when a sample cannot be collected during a flare of symptoms or when other constraints preclude measurement of both molecules.

Although also produced in macrophages[83-86], Langerhans cells[87], liver endothelium[86], platelets[88], Th2 helper T cells[89], stimulated osteoblasts[90], and possibly adipocytes[91], $PGD_2$ appears to be far dominantly produced in MCs[92,93], yielding attractive specificity for clinical detection of MCAD. In patients with MC activation producing increases in urinary *N*-methylhistamine, the fold increase in urinary $PGD_2$ is substantially greater than seen for the histamine metabolite[94]. However, $PGD_2$ appears to have an even shorter half-life than histamine (on the order of 1-30 min in various studies)[95-97]. In fact, $PGD_2$ is metabolized so rapidly that its measured levels may substantially underestimate its total production[98]. Some $PGD_2$ metabolites, though, are more stable than the parent compound (*e.g.*, 9α,11β-$PGF_{2\alpha}$[92]), leading some to preferentially test such metabolites over $PGD_2$. Of note, though, far more $PGF_{2\alpha}$ comes from reduction of $PGH_2$, and even some from $PGE_2$, which are produced by a range of other types of cells[99], making the value of $PGF_{2\alpha}$ as a marker for MC activity less clear). Assays of levels of histamine and prostaglandin metabolites are further complicated by the need of many clinical laboratories to ship samples to a reference laboratory for measurement. Care must be taken by the patient and laboratory staff to maintain the samples (particularly for heparin and $PGD_2$) in chilled condition throughout collection, storage, and transport until final processing. It is unclear whether urinary $PGD_2$ is exclusively a product of the kidneys[100] or dominantly a filtering of plasma $PGD_2$[92]. If the former is true, a low urinary $PGD_2$ could be due to chronic kidney disease (CKD), though certainly patients with CKD due to activated MCs can manifest elevated urinary $PGD_2$ levels. Therefore, CKD is not a reason to forego testing of the urinary $PGD_2$ level.

Ideally, a 24-h urine collection is preferred over a spot/random collection given that the evanescence of mediator flares and the short half-lives of many mediators make spot urine collections and plasma assays less likely to catch elevated levels of mediators. However, from an efficiency perspective, testing both 24-h and random specimens at the same time may not be unreasonable in patients whose mysterious chronic illness has already consumed extensive resources.

Non-steroidal anti-inflammatory drugs (NSAIDs), which inhibit cyclooxygenase and thus limit prostaglandin production, can result in low $PGD_2$ levels in the blood and urine. Similar to the precaution with PPI therapy when assessing for serum CgA elevation, the patient's history of use of NSAIDs, in both prescription and over-the-counter products, should be reviewed when planning the laboratory assessment for prostaglandin levels. Like most PPIs, some NSAIDs have long half-lives permitting daily dosing, and thus patients should be cautioned to abstain, if possible, from all NSAIDs for at least 5 d prior to specimen collection for assessment of prostaglandins.

Given the heterogeneity of MCAD and the above-noted challenges in detecting elevated levels of MC mediators, it should not be surprising that some patients present not only with histories which are classic for MCAS but also with initial screenings for the most sensitive and specific MC mediators which are normal. When repeated efforts to identify aberrant MC activation using the above-described screening approach all fail, consideration can be given to screening for aberrant expression of less specific MC mediators such as Factor Ⅷ[64,101], plasma free norepinephrine[102], tumor necrosis factor alpha[103-111] and interleukin-6[103]. Plasma free norepinephrine is often ordered as part of a plasma free catecholamine profile; in many MCAS patients a pattern of elevated norepinephrine, low-normal or low epinephrine, and sometimes elevated dopamine is seen (LBA and GJM, unpublished data).

Although commercial testing (whether through local or reference laboratories) is not yet widely available, levels of MC mediators leukotriene B4 and cysteinyl leukotrienes C4, D4 and E4 have been reported to be elevated in patients with SM as compared to healthy controls[67]. Plasma and urinary leukotrienes also increase with acute attacks of asthma[112], a disease increasingly suspected to involve aberrantly active MCs[113]. However, leukotriene expression has not been specifically studied in the MCAS population. Furthermore, leukotrienes are synthesized in a variety of myeloid cells (and epithelial cells as well)[114,115], so the specificity of elevated leukotriene metabolite levels for MCAD is unclear and may depend substantially on the presence of a clinical history compatible with MCAD. Whether elevated leukotriene levels are related more to MC load or to MC activity remains to be determined. However, if the clinical history is best explained by MC activation but abnormal levels

of the previously discussed biochemical markers cannot be identified, it would not be unreasonable to also examine levels of plasma and urinary leukotriene metabolites if commercial assays of such are accessible.

## SPECIMEN HANDLING ISSUES

The short half-lives and thermolability of many MC mediators require continuous specimen chilling throughout collection, storage, and transport. Particularly with regard to 24-h urine collections for MC mediator testing, patients should be carefully educated to pre-chill the collection container overnight before beginning the collection and then to keep the container continuously chilled while following an otherwise standard 24-h urine collection protocol; the container should be removed from the refrigerator or ice chest only when imminently needed and should be returned to chilling as soon as possible. Patients should also be cautioned to maintain the container in a chilled environment throughout transport. We recommend the container be placed in a bag filled with ice and sealed, with the bag then placed in an ice chest filled with ice and sealed for transport to the accessioning lab, whereupon the bag can be removed from the chest and provided to the technician with a reminder of the criticality of keeping the specimen chilled.

Laboratory staff often are unfamiliar with MC mediator testing, previously a rarely undertaken endeavor. Consideration should be given by the diagnostician to sharing with laboratory personnel the ultimate clinical goal of such testing and the importance of maintaining thermal integrity of these specimens at all times, including at the time of initial accessioning as well as when packing specimens for transport to reference laboratories that may be thousands of miles distant, transits that may involve long periods sitting in unventilated cargo containers on hot tarmacs. Use of well-insulated containers, and liberal placement of cold packs in the insulated container, should be *de rigueur* when packing such specimens for long-distance transport.

If $PGD_2$ (or 11-β-$PGF_{2\alpha}$) levels below the lower limit of normal are determined and the patient denies any recent use of NSAIDs, or if "normal" MC mediator levels are repeatedly seen despite specimens being accessioned at particularly symptomatic times, it may be useful to ask the patient about his observations, while at the lab, as to whether the laboratory staff maintained the specimen in a chilled environment. Aside from NSAID use, loss of thermal integrity is the most common reason for low $PGD_2$ levels in the authors' experience.

## CONCLUSION

With growing appreciation of the prevalence of MCAD (particularly MCAS), there is a growing need to pursue diagnostic evaluation for MCAD outside of the few centers specializing in this area. An understanding of the spectrum of MCAD and the similarities and differences amongst the various forms of the disease is helpful in guiding the approach to testing. Especially if MCAS is confirmed to usually be a clonal disease, the rise of personal genomics may come to obviate many of the present challenges in diagnosing MCAD, but with appropriate collaboration amongst the diagnostician, patient, other relevant clinicians, and laboratory staff, today's challenges can be surmounted. Especially given that the majority of the MCAD iceberg seems likely to be MCAS and that significantly helpful therapy can be found for most MCAD patients (including most MCAS patients), efforts to smooth the path toward diagnosis of MCAD are worthwhile.

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