RESEARCH REVIEW

Mast Cell Disorders in Ehlers–Danlos Syndrome

SURANJITH L. SENEVIRATNE, ANNE MAITLAND ,* AND LAWRENCE AFRIN

Well known for their role in allergic disorders, mast cells (MCs) play a key role in homeostatic mechanisms and surveillance, recognizing and responding to different pathogens, and tissue injury, with an array of chemical mediators. After being recruited to connective tissues, resident MCs progenitors undergo further differentiation, under the influence of signals from surrounding microenvironment. It is the differential tissue homing and local maturation factors which result in a diverse population of resident MC phenotypes. An abundance of MC reside in connective tissue that borders with the external world (the skin as well as gastrointestinal, respiratory, and urogenital tracts). Situated near nerve fibers, lymphatics, and blood vessels, as well as coupled with their ability to secrete potent mediators, MCs can modulate the function of local and distant structures (e.g., other immune cell populations, fibroblasts, angiogenesis), and MC dysregulation has been implicated in immediate and delayed hypersensitivity syndromes, neuropathies, and connective tissue disorders (CTDs). This report reviews basic biology of mast cells and mast cell activation as well as recent research efforts, which implicate a role of MC dysregulation beyond atopic disorders and in a cluster of Ehlers–Danlos Syndromes, non-IGE mediated hypersensitivity disorders, and dysautonomia. © 2017 Wiley Periodicals, Inc.

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INTRODUCTION: MAST CELLS AND THEIR PROPERTIES

In the late 19th century, Paul Ehrlich named a granule-dense cell, "mast-zellen," situated near blood vessels in the mucosa and connective tissue. He theorized these cells were providing nourishment to the local tissue environment. Using commercial dyes such as dahlia, toluidine blue, methylene blue, and neutral red, he noted metachromatically staining mature mast cells (MCs) in the connective tissue of several organs.

MCs develop from multipotent hemopoietic progenitors in the bone marrow [Moon et al., 2010]. Stem cell factor (KIT ligand) binds to homodimeric KIT (a transmembrane tyrosine kinase receptor) and influences MC differentiation, growth, survival, migration, and effector functions. MCs acquire a tissue specific phenotype depending on signals they receive from the local tissue environment. Several factors such as interleukin-3 (IL-3), interleukin-4 (IL-4), interleukin-9 (IL-9), and transforming growth factor β1 (TGFβ1) have been shown to influence the number and mediator content of MCs [Galli et al., 2011].

Under non-pathological states, mature differentiated MCs are found exclusively within tissues, compared to other innate immune cells, such as basophils, neutrophils, and eosinophils. Within tissues, MCs congregate around nerves, blood vessels, and lymphatic vessels. Based on their location (connective tissue or mucosal) and content of their granules, two types of MCs have been described. MCs residing in connective tissue, skin, and the peritoneal cavity contain tryptase (MC_T) in their granules and express interleukin-5 (IL-5) and interleukin-6 (IL-6). MCs homing to the gut and respiratory mucosa contain tryptase and chymase (MC_{TC}), and express IL-4 [Sigal, 2011]. When fully differentiated, MCs exhibit a wide range of biological properties including phagocytosis, antigen presentation, cytokine and chemokine production, and the immediate release of vasoactive substances. They have a role in local tissue homeostasis (tissue repair, angiogenesis) and co-ordination of immune responses to a myriad of pathogens, recognized through evolutionarily conserved surface receptors like toll-like receptors, complement receptors, and receptors for adenosine phosphate, oestrogen, and immunoglobulins), physical stimuli (pressure, temperature), and toxins. As yet, no animal model or disease state has

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been identified where there is a complete lack of MCs [Metcalfe et al., 1997]. When activated, MCs produce a range of preformed and newly synthesised mediators (Fig. 1) [Louisias et al., 2013]. Within minutes of activation, preformed mediators (histamine and proteases) are released. This is followed by de novo synthesis of membrane-derived lipid mediators (prostaglandins and leukotrienes) and a range of pro- and anti-inflammatory cytokines and chemokines.

MCs residing in connective tissue, skin, and the peritoneal cavity contain tryptase (MC_T) in their granules and express interleukin-5 (IL-5) and interleukin-6 (IL-6).

MCs are best known for their role in immediate IgE-mediated, allergic responses in anaphylaxis, food allergy, venom allergy, and asthma. Recent reports have also implicated MCs in nonallergic disorders, including headache syndromes, irritable bowel syndrome, non-celiac gluten enteropathy, osteoporosis, autoimmune syndromes, neuropsychiatric disorders, and interstitial cystitis [Theoharides et al., 2015].

MAST CELLS AND CONNECTIVE TISSUE

Different components of the extracellular matrix affect the migration and differentiation of MC progenitors, MC activation, and pattern of mediator release. Human MCs express laminin receptors and can adhere to fibronectin and vitronectin.

The hypermobile type of Ehlers–Danlos Syndrome (hEDS) is the dominant form of EDS. A subpopulation of hEDS patients have been found to have MCAD (more often MCAS than SM). Several reports have described of co-morbid clinical manifestations in patients with CTDs, including EDS, functional gastrointestinal disorders [Fikree et al., 2015]; eosinophilic gastrointestinal disorders [Abonia et al., 2013]; an increased prevalence of asthma [Morgan et al., 2007], neuropsychiatric conditions [Sinibaldi et al., 2015], and osteoporosis [Deodhar and Woolf, 1994]; and orthostatic intolerance [Garland et al., 2015]. Luzgina et al. [2011] found an increased number of chymase-positive MCs in the eyelid skin of patients with CTDs (joint hypermobility, skin hyper-elasticity, spinal deformities, thumb and wrist sign, vascular, fragility, varicose veins, and telangiectasias).

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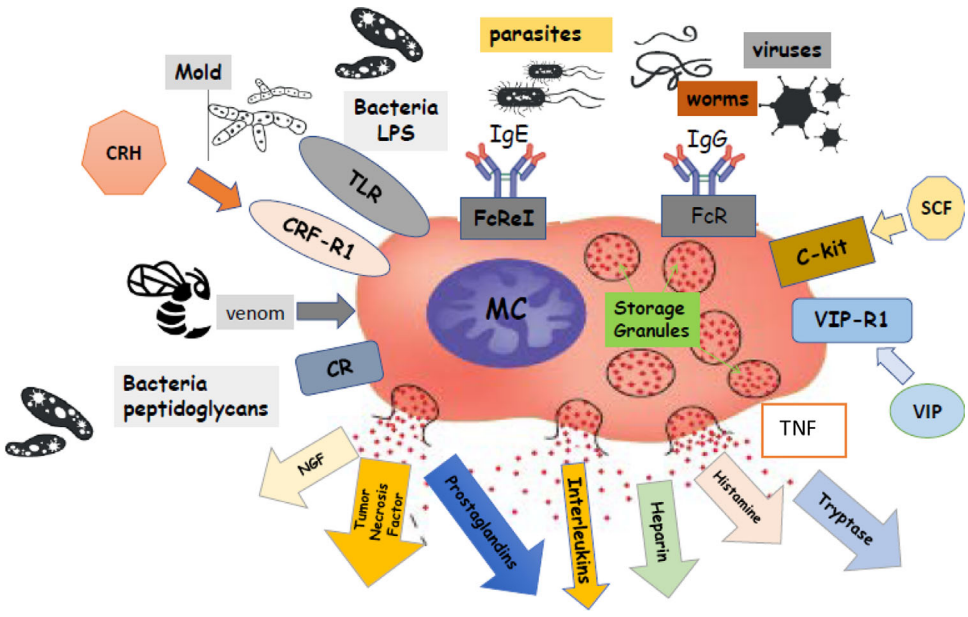

Figure 1. MC activate and release bioactive substances, responding to a variety of mechanical, biological, or chemical stimuli. MCs release preformed mediators in granules (histamine, heparin, serotonin, and enzymes) and newly synthesized (cytokines, growth factors, and lipid metabolites). C-kit, transmembrane tyrosine kinase receptor; CR, complement receptor; CRF-R1, corticotropin releasing factor receptor 1; CRH, corticotropin releasing hormone; FcεRI, Fc epsilon receptor I; FcγRI, Fc gamma receptor I; LPS, lipopolysaccharide; MC, mast cell; NGF, nerve growth factor; SCF, stem cell factor; TLR, Toll-like receptor; TNF, tumor necrosis factor-α; VIP, vasoactive intestinal peptide; VIP-R1, vasoactive intestinal peptide receptor 1. Adapted from the following references: [Afrin, 2013; Cardet et al., 2013; Theoharides et al., 2015] and arranged using images from shutterstock.com.

EDS, functional gastrointestinal disorders; eosinophilic gastrointestinal disorders; an increased prevalence of asthma, neuropsychiatric conditions, and osteoporosis; and orthostatic intolerance.

Several investigators have noted a possible link between EDS and MCAD, primarily patients with the hypermobility type of EDS. Immunohistochemisty analysis identified an increased content of chymase positive MCs in undamaged skin of patients with signs suggestive of CTDs (hyperelasticity of the skin, joint hypermobility, spine and thorax deformities, thumb sign, wrist sign, vascular fragility, varicose veins, and telangiectasias) [Luzgina et al., 2011]. Louisias et al. [2013] described symptoms compatible with a non-IgE mediated MC disorder in patients with the joint hypermobility syndrome: most reported naso-ocular symptoms, asthma, and history of anaphylaxis and describe a positive response to classical MC/MC mediator antagonists. Plasma histamine and serum tryptase levels were normal and prostaglandin measurements were not undertaken. Cheung and Vadas [2015] suggested a possible new disease cluster: Mast Cell Activation Syndrome (MCAS), Postural Orthostatic Tachycardia Syndrome (POTS), and EDS. Patients having a diagnosis of POTS and EDS were given a screening questionnaire to look for symptoms consistent with MCAS, and 66% of the respondents reported such symptoms. Recently, Milner et al. identified families with an elevated, baseline serum tryptase, which was associated with the triad of dysautonomia, MCAD, and joint hypermobility [Lyons et al., 2016]. The elevated tryptase level was not consistent with SM. Instead, increased copy numbers of the *TPSAB* gene, that encodes alpha tryptase, were detected. Moreover, these observations highlight the role of MCA, impacting the structure and function of connective tissue, as described in inflammatory arthritis [Nigrovic and Lee, 2005].

MAST CELL ACTIVATION DISORDERS

Mast cell activation disorder (MCAD) refers to an increased number of MCs, increased activity of MCs, or both. Akin et al. [2010] classified diseases associated with MC activation as primary, secondary, and idiopathic groups (Table I). The conditions may be associated with (1) "an expansion of clonal MCs," and/or (2) by increased, aberrant MC mediator release. Monoclonal MC activation syndrome (MMCAS) was included within the primary group; non-clonal MC activation syndrome (MCAS) was included within the idiopathic group.

Consensus diagnostic criteria have been established for most of the forms of mastocytosis (e.g., the WHO 2008 criteria define the approach to systemic mastocytosis (SM) [Horny et al., 2008]. However, as MCAS is so recently recognized, no consensus definition has yet been established. There are two proposals for diagnostic criteria for MCAS [Molderings et al., 2011; Valent et al., 2012] (Table II). The presence of EDS (of any form) in the patient's history is not known to affect the approach to diagnostic evaluation for MCAD.

The most well-known form of MCAD, MC disorders proven to be primary/clonal are rare, with an estimated prevalence of one case per 10,000–100,000 persons. Primary, clonal MC disorders include mastocytosis and MMCAS. Reported secondary causes of MC disorders include comorbid immune disorders, including classic atopic syndromes ("allergies"/IgE-Fcε receptor-mediated MC activation); autoimmune disorders (autoimmune chronic urticaria, multiple sclerosis, rheumatoid arthritis) [Benoist and Mathis, 2002]; and chronic infections, of which some likely occur in the context of primary immune deficiency disorders [Cardet et al., 2013].

Allergic disorders are a well-recognized cause of MC activation (MCA). Here, allergens cross link IgE molecules on the surface of MCs, leading to MCA,

TABLE I. Classification of Diseases Associated With Mast Cell Activation (Adapted From [Akin, 2014; Theoharides et al., 2015])

Primary	Mastocytosis
	Monoclonal Mast Cell Activation Syndrome
Secondary	Allergic/atopic (IgE mediated) disorders
	Mast cell activation associated with chronic inflammatory or neoplastic disorders
	Physical urticarias
	Chronic autoimmune urticaria
Idiopathic	Anaphylaxis
	Angioedema
	Urticaria
	Mast cell activation syndrome

TABLE II. Diagnostic Criteria for Systemic Mastocytosis and Mast Cell Activation Syndrome (Adapted From Afrin, World Journal of Haematology, 2014)

WHO 2008 diagnostic criteria for systemic mastocytosis
Major criterion
Multifocal dense aggregates of MCs (15 or more) in sections of bone marrow or other extra-cutaneous tissues and confirmed with tryptase immunochemistry or other special stains
Minor criteria
Atypical or spindled appearance of at least 25% of the MCs in the diagnostic biopsy
Expression of CD2 and/or CD25 by MCs in marrow, blood, or extra-cutaneous organs
KIT codon 816 mutation in marrow, blood, or extra-cutaneous organs
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 criterion for MCAS: Valent et al. [2012] criteria
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
Absence of any other known disorder that can better account for these symptoms
Increase in serum total tryptase of 20% above baseline plus 2 ng/ml during or within 4 hr after a symptomatic period
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. [2011] criteria
Major criteria
Multifocal MC aggregates as per WHO major criterion for SM
Clinical history consistent with chronic/recurrent aberrant MC mediator release
Minor criteria
Abnormal MC morphology as per WHO SM minor criterion 1
CD2 and/or CD25 expression as per WHO SM minor criterion 2
Detection of known constitutively activating mutations in MCs in blood, marrow, or extracutaneous organs
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

TABLE III. 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

A: Clinical signs & symptoms of mastocytosis patients. Adapted from [Alvarez-Twose et al., 2010]	
Sign/symptom	%
Skin lesions	90
Pruritus	82
Flushing	56
Diarrhoea	35
Abdominal cramping	30
Neuropsychiatric symptoms	23
Anaphylactic	23
Peptic symptoms	20
Osteoporosis	18
Hepatomegaly	12
Splenomegaly	08

B: Clinical signs & symptoms of "Non-clonal" mast cell activation disorder. Adapted from [Hamilton et al., 2011]	
Sign/symptom	%
Abdominal pain	94
Dermatographism	89
Flushing	89
Headache	83
Neuropsychiatric symptoms	67
Diarrhoea	67
Rhinitis	39
Asthma	39
Anaphylaxis	17

releasing a range of mediators and producing the well-known range of allergic manifestations. Physical MC triggers constitute common, non-IGE MCADs, such as those affected by physical urticarias, including cholinergic and cold-induced urticaria [Simons, 2010]. Research efforts are now describing mechanisms of non-IGE mediated MCA. Oxidative mechanical stress has been shown to induce MC mediator release [Briganti et al., 2001]. Boyden et al. [2016] described a missense variant in *ADGRE2* associated with vibratory urticaria. Recently, in subjects with an inherited, elevated baseline of serum tryptase, Milner et al. described a dominant inheritance of increased copy number of *TPSAB1* gene, which encodes alpha tryptase. Clinical manifestations of hypertryptasemia include dysautonomia, joint hypermobility, and MC activation [Lyons et al., 2016]. Active research is also exploring the neurohormonal activation of MCs [Theoharides et al., 2015]. If a clonal MC disorder is not detected nor a secondary cause identified, as detailed above, then children and adults with clinical symptomology and with evidence of aberrant MC mediator release are deemed to have idiopathic MCAD [Cardet et al., 2013; Picard et al., 2013; Theoharides et al., 2015].

CLINICAL FEATURES OF MCAD

The clinical presentation of MCAD tends to be very heterogeneous. Clinicians need to be aware of this so as to suspect the condition and carry out appropriate testing. Mutational heterogeneity in the affected MC subsets may contribute to the heterogeneity of clinical expression. Common presenting symptoms and signs of mastocytosis and MCAS are given in Table III [Alvarez-Twose et al., 2010; Hamilton et al., 2011]. Conditions that need to be considered in the differential diagnosis of MCAD include: cardiovascular, endocrine, gastrointestinal and neurological disorders, infections, and medication-induced side effects.

The following criteria have been proposed for diagnosing MCAD:

(1) Typical signs and symptoms of MC mediator release (affecting at least two organ systems)
(2) [Akin, 2014; Theoharides et al., 2015]

Skin	Flushing, pruritis, urticaria, angioedema
Cardiovascular	Hypotension
Respiratory	Asthma: cough, wheezing; throat swelling
Gastrointestinal	Diarrhea, bloating, cramping
Naso-ocular	Rhinitis, pruritis
Anaphylaxis	Stinging insect allergy, peri-operative anaphylaxis

(3) Objective evidence of MC-derived mediator release or chronically activated MCs [Afrin, 2013; Cardet et al., 2013]

Tryptase**	Baseline elevated level; Elevated serum tryptase, following a suspected MC activation event: 20% + 2 ng/ml above baseline
Histamine	Elevated 24-hr urinary histamine metabolite (N-methylhistamine)
Prostaglandin	Elevated 24-hr urinary prostaglandin D_2; 11-β-prostaglandin-$F_{2\alpha}$
Tissue Biopsy	CD117 + cells that are clustered and/or, spindle-shaped; co-expression of CD25 and CD2 on CD117 + Cells (MCs)
Heparin	Increased blood level
Chromogranin A	Increased blood level (note confounders of cardiac or renal failure, proton pump inhibitor use, or neuroendocrine cancer)

**Although all MCs contain tryptase, there is evidence indicating that tryptase is not always secreted following engagement of different MC activation pathways [Marshall, 2004].

(4) Response to therapy that directly or indirectly blocks MC mediator activity [Cardet et al., 2013; Akin, 2014]

Histamine H_1 and H_2 receptor blockade
Ketotifen
Cromolyn sodium
Aspirin
Leukotriene receptor antagonists
Omalizumab

A diagnostic algorithm for MCAD is shown in Figure 2 [Picard et al., 2013].

LABORATORY ASSESSMENT OF MCAD

Diagnostic pursuit of MCAS typically focuses on probing blood and urine for elevated levels of mediators relatively specific to the MC [Afrin and Molderings, 2014]. However, at present, of the over 100 mediators produced by activated MCs, only handful can be measured within commercial laboratories. Assays certified for clinical use are not available for most MC mediators, and even for those MC mediators which can be tested in the clinical laboratory, most have an unfavorable level of specificity for the MC, leaving relatively few mediators to be tested. Furthermore, many of the diagnostic tests are not widely available to the clinician or are cost-prohibitive. Ideally, serum tryptase and chromogranin A, plasma histamine, prostaglandin (PG)D_2, and heparin, as well as urinary (random and 24-hr) histamine, *N*-methylhistamine (NMH), PGD_2, 11-β-$PGF_{2\alpha}$, and leukotriene (LT) E4 should be assessed. Whether to pursue such tests in parallel or sequentially depends on the balance between diagnostic expediency versus containment of testing costs.

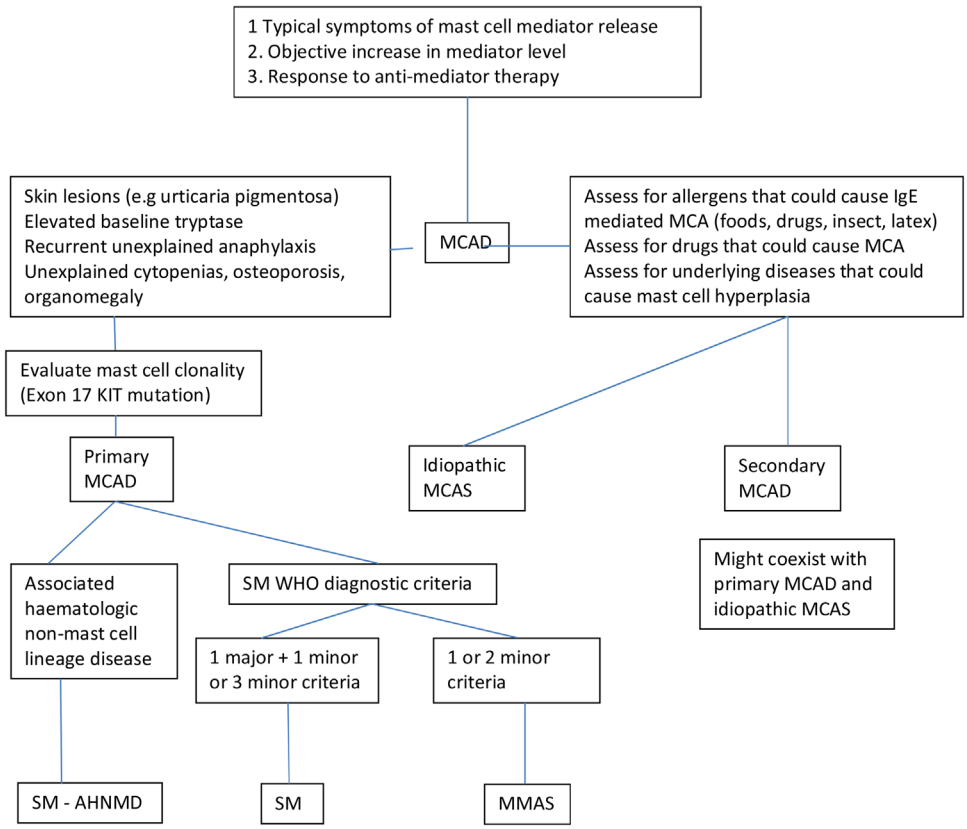

Figure 2. Diagnostic algorithm for mast cell activation disorders. Permission to reprint from Dr. Mariana Castells, [Picard et al., 2013]. KIT, a transmembrane type III tyrosine kinase receptor; AHNMD, associated hematologic non-mast cell lineage disease; MCA, mast cell activation; MCAD, mast cell activation disorder; MCAS, mast cell activation syndrome; MMAS, monoclonal mast cell activation syndrome; SM, systemic mastocytosis.

Some metabolites have longer half-lives (e.g., tryptase, histamine) and are thermostabile than others (e.g., heparin, the prostaglandins). It is prudent to continuously chill all specimens throughout collection and handling (including centrifugation). The authors recommend identifying at least two elevated MC mediator levels (either the same mediator, or different mediators), and preferably across two different time points (in keeping with the chronic clinical nature of the disease), before diagnosing MCAS in a patient with a history consistent with chronic/recurrent aberrant MC mediator release and absence of any other evident disease better accounting for the full range and chronicity of all the symptoms and findings in the past.

The technical and logistical challenges in MC mediator testing need to be acknowledged. If mediator testing is negative in a patient whose clinical history strongly suggests MC activation, repeat testing should be done at a timepoint when the patient is particularly symptomatic. Upper and/or lower gastrointestinal tract mucosal biopsies stained for MCs (CD117 at a minimum) looking for increased numbers of or constitutively active MCs also can be helpful.

Serum Tryptase Levels. Tryptase is the most abundant protein in MCs and is heat stable. The amount found in basophils is about 300 times lower than in MCs. The human tryptase gene is located on chromosome 16, and codes for five isoenzymes: alpha, beta, gamma, delta, and epsilon. Beta tryptase is the predominant form stored in the MC granule. It is found as a tetramer and is stabilized by proteoglycans such as heparin. The isoforms are continuously released from MCs into the bloodstream and basal levels are a reflection of total MC numbers [Schwartz, 2006]. The ImmunoCAP® Tryptase assay measures total tryptase levels (that is all inactive proforms of alpha-tryptase and beta-tryptase, as well as the enzymatically active mature beta-tryptase). The conditions/disorders that cause an elevated basal serum tryptase level are given in Table IV.

Basal serum tryptase levels of 20 ng/ml are considered as a decision point in several MC diagnostic criteria. For example, a basal serum tryptase level greater than 20 ng/ml is a minor diagnostic criterion for SM. Valent et al. [2012] suggested a rise in serum total tryptase of 20% above baseline, plus 2 ng/ml, within 4 hr of the onset of an acute flare of symptoms as a

TABLE IV. Conditions/Disorders That Could Cause Elevated Basal Serum Tryptase Levels Adapted From the Following References [Cardet et al., 2013; Picard et al., 2013]

Condition/disease	Source of tryptase
SM	Neoplastic MCs
MCAS	Activated MCs (monoclonal or polyclonal)
Allergic/atopic disorders	Activated MCs and/or activated basophils
End stage kidney disease	Normal MCs
Helminth infection	Reactive MCs
Myelodysplastic syndromes	Neoplastic MCs or/and basophils or/and blast cells
Acute myeloid leukaemia	Myeloblasts
Chronic myeloid leukaemia	Immature leukaemic basophils (rarely MCs)
Chronic eosinophilic leukaemia	Neoplastic MCs
Idiopathic	Unknown

Some haemodialysis patients may present with elevated tryptase levels, due to reduced excretion.
MC, mast cell; MCAS, mast cell activation syndrome; SM,systemic mastocytosis.

significant rise. However, as yet there has not been clinical validation of this formula as a discriminating tool for diagnosing MCAS.

It needs to be stressed that a normal serum tryptase level does not exclude MCAS. Furthermore, levels above 20 ng/ml do not exclude MCAS, and levels below 20 ng/ml do not exclude SM. Persistence of the serum tryptase level above 20 ng/ml makes SM more likely, necessitating marrow examination to exclude mastocytosis. Even if the serum tryptase level is persistently below 20 ng/ml, consideration of SM may need to made if the patient's history of illness is more consistent with SM (i.e., sudden onset of symptoms in middle or older age in contrast to MCAS's usual history of symptoms dating back to adolescence or childhood).

Urinary or Plasma Histamine and Histamine Metabolites

Levels are measured in a 24 hr urine collection, a spot urine sample and plasma sample. Sample chilling is important and special care needs to be taken with sample collection and storage. The levels are best measured during an attack or soon thereafter and should be undertaken within a laboratory with experience in their measurement.

Prostaglandins and leukotrienes in urine or plasma: Prostaglandin (PGD2), 11-β-prostaglandin-$F_{2\alpha}$, or leukotriene (LTE4) are measured in plasma or a 24-hr urine sample [Schäfer et al., 2014]. The patient needs to take care in keeping the sample chilled when collecting a 24 hr urine sample. PG interpretation is confounded by recent use of non-steroidal anti-inflammatory drugs (NSAIDs). When NSAIDs have not been recently used, the finding of PG levels below the lower limit of normal may point toward the loss of the sample's thermal integrity while en route to the reference laboratory.

Histopathological Examination of Bone Marrow

A bone marrow biopsy needs to be considered when the baseline tryptase level is >20 ng/ml or those who have syncopal or pre-syncopal events as part of their symptoms (irrespective of the tryptase levels) [Akin, 2014]. Such an examination may need to be bilateral to increase the chances of finding the typically patchily distributed aggregates of abnormal MCs [Butterfield and Li, 2004].

In addition to evaluating the number of MCs, changes in morphology or distribution and evidence of degranulation should be looked for. Antibodies against KIT (i.e., CD117), CD25, tryptase, and chymase should be used. Flow cytometric assessment for co-expression of CD117 together with CD25 and/or CD2, CD30, and mutational assessment for mutations in KIT (in particular, the *KIT* D816V mutation at a minimum) should be done. CD25 expression in MCs is a minor diagnostic criterion in SM. When MCAS seems more likely than SM, marrow examination is usually unhelpful. Even if MMCAS is found on flow cytometric or mutational testing, at present there are no known differences in prognosis of, or the therapeutic approach toward, MMCAS versus MCAS.

Histopathological Examination of Other Tissues

If mediator testing is unrevealing, the Molderings diagnostic criteria (REF) provide an alternative path to diagnosis of MCAS, namely, finding increased MCs in extracutaneous tissue, most commonly gastrointestinal (GI) or genitourinary (GU) tract mucosal biopsies. Biopsy specimens obtained from the gastrointestinal tract, urinary bladder, and skin should be stained for mast cells. A cut-off of >20 MCs per high power field has been considered by several groups when interpreting the tissue biopsy findings. Traditionally, staining of tissue biopsies for MC disease has employed stains targeting MC granules or their contents, for example, tryptase, Giemsa, toluidine blue, Alcian blue, etc.

However, more recently, it has become clear that CD117 (the dominant MC regulatory element brightly present on virtually all MCs) more reliably reveals MCs [Feyerabend et al., 2005; Leclere et al., 2006]. Given that the essence of MCAD is inappropriate MCA and thus inappropriate MC degranulation, relying on granule-targeting stains may increase the false negative rate. MCs in SM are found in abnormal aggregates but remain normally dispersed in MCAS. Furthermore, the MCs in SM typically are of aberrant morphology (most commonly spindled) while in MCAS they retain their normal round to ovoid shape. Again, CD117 co-expression with CD25 and/or CD2 is uncommonly found on flow cytometry in MCAS (whether in marrow or other tissue), and *KIT* codon 816 mutations, too, are rarely found in MCAS.

Gene Mutation Analysis

Although *KIT* D816V mutation testing by routine PCR analysis in the blood of SM patients is often positive (but far from perfect), this testing is virtually always negative in the far more prevalent setting of MCAS. Real-time quantitative PCR testing for *KIT* D816V is far more sensitive and has been shown in at least one study to be positive in 100% of SM patients [Kristensen et al., 2011]. There have been no reports that investigated this technique in the MCAS population.

Molderings [2015] have published two studies showing that on "full sequencing" of MC *KIT*, although one or more mutations are found in almost every MCAS patient, codon 816 mutations (whether D816V or any other) are virtually never found in MCAS. Clearly, codon 816 mutations have consequences that strongly influence the development of MCAD toward the mastocytosis phenotype. Without codon 816 mutations, it is far more likely MCAD will develop toward the MCAS phenotype. Although testing for this mutation on a bone marrow sample is more appropriate in SM, some patients do not wish to undergo the more invasive procedure.

Chromogranin A (CgA)

CgA is a heat-stable, 439-amino acid protein, and a member of the granin family of proteins. Granins are widespread in endocrine, neuroendocrine, peripheral, and central nervous tissues, where they are found in secretory granules. CgA is also secreted by MCs. It is known to be elevated in heart and renal failure, neuroendocrine cancer, and when proton pump inhibitors (PPIs) are being used. PPI therapy should be omitted for at least 5 days prior to measurement of baseline levels.

Plasma Heparin

Heparin may be the single best performing diagnostic marker for MC activation [Vysniauskaite et al., 2015]. However, the level of endogenous plasma heparin found normally and even in most cases of MCAS is below the lower limits of detection of most clinical assays for this metabolite (typically 0.10–0.30 anti-Factor Xa units/ml). Standard clinical assays are engineered to assist with monitoring of heparin therapy, which produces far higher levels of heparin than found normally or in MCAS. Thus, although an occasional MCAS patient may present a level detectable by one of the commonly used assays, typically a more sensitive assay is needed. As the half-life of heparin is approximately 1 min (similar to PGD_2), sample chilling is important.

TREATMENT OF MCAD

MCAD is presently incurable (except for the rare instance of a solid mastocytoma) and therapy, is therefore, symptomatic except when cytoreduction is additionally required in advanced mastocytosis. MCAD therapy should always include maneuvers aimed at controlling MC mediator production, release, and end-organ effects.

Many cases of childhood cutaneous mastocytosis (CM) seem to spontaneously regress in adolescence. However, it seems that MCAS emerges in at least some such patients within several years after regression of CM (personal observation, LBA). Only in the relatively rare forms of SM which either are aggressive malignancies themselves (e.g., aggressive SM or MC leukemia) or are associated with significant malignancies (e.g., SM with associated clonal hematologic non-MC-lineage disease, or SM-AHNMD in the WHO 2008 classification) are cytotoxic/chemotherapeutic and cellular therapies considered. Such therapies have been extensively discussed elsewhere in the literature and are beyond the scope of this paper.

In general, co-morbidity of EDS (any form) is not known to affect the approach to treatment of MCAD, except to note that chronic glucocorticoid therapy (a poor choice anyway in MCAD given the treatment's chronic toxicities, including in connective tissues) may be an even poorer choice in MCAD patients also featuring EDS. Desensitization therapy can be considered. It is important that patients identify potential triggers for their symptoms (dietary, chemicals, medications, allergens), and environmental modifications, to reduce exposures. Common MC triggers are given in Table V. MCAD patients have many physical sensitivities (e.g., heat, cold, ultraviolet radiation, exertion, etc.) and antigenic sensitivities (e.g.,pollen, mold, etc.). There is a core group of foods (tending to be patient-specific) that many patients find difficult to consume without developing adverse symptoms.

In general, co-morbidity of EDS (any form) is not known to affect the approach to treatment of MCAD, except to note that chronic glucocorticoid therapy (a poor choice anyway in MCAD given the treatment's chronic toxicities, including in connective tissues) may be an even poorer choice in MCAD patients also featuring EDS.

TABLE V. Triggers of Mast Cell Activation Adapted From the Following References [Cardet et al., 2013; Picard et al., 2013]

Alcohol
Heat
Drugs: antibiotics, NSAIDs (nonsteroidal anti-inflammatory drugs), Narcotics, Neuromuscular blocking agents
Radiocontrast media
Invasive procedures (e.g., general anaesthesia, biopsy, endoscopy)
Hymenoptera stings
Fever or infection
Exercise
Physical stimuli (e.g., pressure, friction)
Emotions/stress

Patients and clinicians should be alert to the propensity of MCAD patients to react to medication excipients. The emergence of adverse reaction within the first few doses of an ordinarily well tolerated medication should prompt (1) a review of the formulation's ingredient list to try to identify a particular offending excipient; and (2) identification of alternative formulations to be tried, containing as few of the excipients in the offending formulation as possible. Sometimes MCAD patients benefit from custom-compounded formulations of their medications.

Some MCAS patients are highly reactive to a range of foodstuffs. Elimination diets such as described for the eosinophilic esophagitis population are helpful in some patients but not others. As with medication trials, diet trials typically need to last only 1–2 months to determine if they are going to be significantly beneficial. The implementation of more than one change around the same time (e.g., a dietary change around the same time as a medication change) can be greatly confounding and should be avoided.

In spite of the substantial fatigue and malaise that many MCAD patients experience, they should be strongly encouraged to exercise regularly. This should only be to the usual individual limit of tolerance that each patient has likely learned from experience. This is because overexertion could trigger a flare of MC activation in some patients. Since both physical and psychological stress have long been known to activate MCs, interventions aimed at stress reduction (e.g., psychotherapy) can be helpful.

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An effective "primary" physician—whether a primary care physician or specialist—is critically important for successful management of most complex diseases, including MCAD. The absence of a local "physician/partner" who can reliably help the patient access local health care resources as needed and remote resources could lead to the MCAD patient facing difficulty in gaining and then maintaining control over their disease.

Drug treatment needs to be tailored to the individual patient as their tolerance and the symptomatic benefit they receive tends to vary. Commonly used medications include H_1 and H_2 antihistamines, sodium cromoglicate, ketotifen, omlaizumab, and the leukotriene receptor blockers. Medications should usually be added one at a time, with an adequate time interval between the additions of successive drugs. Some patients need to begin medications at a lower dose and then gradually escalate to a standard dose. Patients need to be told that the time for noticing an initial symptomatic response may be a few weeks [Cardet et al., 2013; Akin, 2014; Zhang et al., 2016].

Many agents have been shown to significantly help various MCAD patients, but at present therapeutic response profiles appear highly individualized. There are no biomarkers predictive of response in general or of which symptoms will respond to any given agent in a given patient.

H_1 and H_2 Antihistamines

These medications block the H_1 and H_2 receptors present on many end organs and on MCs themselves, too. They have been in use for many years and most doctors are aware of their beneficial effects and potential adverse effects. Longer acting, generally non-sedating second generation H_1 antihistamines (e.g., cetirizine, fexofenadine, loratadine) have been used in preference to the older, sedating H_1 antihistamines. Most patients need a higher dose (between two to four times) the dose

used for treatment of mild hay fever symptoms. Many patients find a 2–3 times daily dosing to be more helpful than a once daily dosing regimen. The H_2 antihistamines (e.g., ranitidine, famotidine) are helpful for abdominal symptoms and sometimes benefit extra-gastrointestinal symptoms, too. Antihistamines need to be taken for an adequate length of time. Patients should be discouraged in making frequent changes to the doses they take.

Sodium Cromoglicate

Many patients add the MC-stabilising drug sodium cromoglicate to their H_1/H_2 antihistamines, with a view to getting additional symptomatic benefit. It is important that mediator measurements are done prior to this medication been added. Some patients experience a flare of symptoms during the initial few days of taking this drug. Oral, liquid, inhaled, and ophthalmic formulations are available. Although the drug is poorly absorbed and undergoes little systemic circulation, there is a systemic (IV) formulation in development.

Ketotifen

This has both MC-stabilising and antihistamine effects. A few patients are not able to tolerate this because of drowsiness. Tablet, liquid, and eye drop formulations are available. Oral ketotifen is an inexpensive drug, but its availability in the United States only in compounded form increases its expense there.

Leukotriene Receptor Blockers (e.g., Montelukast)

This is a widely used medication in asthma and spontaneous chronic urticaria patients. It is generally well tolerated. Administration twice daily may benefit MCAD patients more than once daily.

Steroids

The long-term use of oral steroids at any dose is discouraged due to well-known toxicities. In addition to its many adverse effects, its effect on bone density would not be helpful in patients with a disorder of connective tissue such as EDS. However, the use of a short course of steroids may be needed if there is acute onset of skin or airway reactivity. Low dose inhaled steroids may be needed if airway hyper-reactivity is present.

Self-Injectable Epinephrine Devices

All patients with systemic MC activation or susceptible to anaphylaxis should be prescribed two self-injectable epinephrine devices and taught how and when this should be used. A glucagon autoinjector may be needed instead if the patient requires beta adrenergic receptor blockade.

Other Medications

From the few reports available, non-steroidal immunosuppressants such as cyclophosphamide, cyclosporine, azathioprine, and monoclonal antibodies such as omalizumab [Zhang et al., 2016] and alemtuzumab are only occasionally helpful [Afrin, 2013]. Several patients find a range of other preparations (such as vitamin C, aspirin, flavone analogues, cannabinoids, etc.) to help their symptoms. Low-dose hydroxyurea helps some MCAD patients and is safely used for years to decades—indeed, life-long—in certain other diseases. A wide range of supportive medications are used by the MCAD population including decongestants, bronchodilators, antiemetics, proton pump inhibitors, anti-depressants of various classes (e.g. tricyclic agents), bowel motility agents, micronutrient supplements, pancreatic enzyme supplements, bone-strengthening agents such as bisphosphonates, tumor necrosis factor (TNF) alpha antagonists, etc.

Next to fatigue, pain is one of the most common symptoms of MCAD. NSAIDs help some patients but are triggers (potentially to anaphylactic extent) in others and must be initiated cautiously when no history of NSAID tolerance is known. Narcotics, too, commonly are triggers; fentanyl, tramadol, and hydromorphone tend to be better tolerated than other narcotics in MCAD patients. Sometimes other classes of MC-targeted agents typically without analgesic effect nevertheless prove analgesic (e.g., antihistamines may relieve chronic migraine headaches in some MCAS patients).

Given the rarity of SM, together with how recently MCAS has come to be recognized, there are no large controlled studies of any intervention for MCAD. Few clinical trials in SM have been performed, and there have been no clinical trials yet in MCAS. Patient and treating clinician alike must take a methodical approach in stepping through trials of the many therapies shown helpful in various MCAD patients, limiting to one change at a time in the regimen whenever possible. Most such treatment trials need last only 1–2 months, typically starting at low doses and escalating step-wise as tolerated to identify maximal effective dosing. Clearly significantly effective treatments are retained, while others not meeting that high bar are stopped lest unmanageable polypharmacy develop. In the absence of a more scientifically informed strategy at present, proceeding in order of treatment cost often is the most reasonable approach.

The most inexpensive and sustainable therapy for MCAD includes the histamine H_1 and H_2 receptor blockers. Benzodiazepines, NSAIDs including aspirin (in patients that can tolerate them), flavonoids (such as quercetin and luteolin), alpha lipoic acid, N-acetylcysteine, and Vitamin C are also inexpensive interventions. Leukotriene receptor blockers and synthesis inhibitors are somewhat more expensive, as are sodium cromoglicate, pentosan, and cannabinoids. Emergency and perioperative management of severe flares of mast cell disease has been amply discussed in the literature and is available publicly [Akin, 2014]. In general, histamine H_1 and H_2 receptor antagonists, glucocorticoids, and benzodiazepines form the core of the therapeutic attack at such a problem.

The clinical (and suspected underlying mutational) heterogeneity of MCAD ensure each therapy found helpful in certain patients will fail in others. Thus, failure of any given therapy (even antihistamines) should not be taken as a sign of

either misdiagnosis or "refractory" disease. With sufficient persistence at trying various interventions, most patients with non-malignant MCAD can eventually identify a regimen that helps them achieve the presently subjective goal of feeling significantly better than the pre-treatment baseline the majority of the time.

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