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How to diagnose Mast Cell Activation Syndrome? Practical considerations.

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Abstract

Mast cells (MCs) are important component of the immune system. Their physiological function is related to multiple areas of human physiology, thus symptoms of their increased activation vary greatly from severe allergic reactions such as anaphylaxis, to chronic symptoms like depression or osteoporosis. Studies on mastocytosis revealed a subgroup of patients presenting symptoms of increased degranulation of MCs, defined as Mast Cell Activation Syndrome (MCAS). Among them patients with primary MCAS presenting clonal abnormal MCs, who do not fulfill the criteria of mastocytosis. These symptoms often overlap patients comorbidities increasing difficulty of MCAS diagnosis and treatment. The diagnosis is established based on 3 criterions: 1) typical symptoms, 2) serum tryptase elevation and 3) response to anti-mediator treatment. MCAS diagnosis is important especially in patients with anaphylaxis, osteoporosis who require epinephrine emergency kit and insect venom immunotherapy. The article describes genetic mechanisms, typical symptoms, criteria and diagnose implications of MCAS. The emphasis is put on practical guidance with the aim to improve patient care by practicing physicians.
Introduction

Mast cells belong to important cells of the immune system. They were described by Paul Ehrlich German scientist born in Strzelin in Lower Silesia who was awarded with a Noble Price for his contribution to immunology.

The physiological function of mast cells is related to several areas of human physiology. Mast cells are main effector cells in type I allergic reactions and diseases like asthma, allergic rhinitis and conjunctivitis, urticaria and anaphylaxis (1). The binding of allergen to IgE connected with FcεRI on the mast cell surface lead to the degranulation and release of the mast cell mediators (1).

Clinical symptoms caused by local or systemic mast cell activation are present in skin (flushing, pruritus, urticaria, angioedema), respiratory system (nasal congestion, nasal pruritus, wheezing, throat swelling, hypoxemia, cough, stridor), circulation (hypotension, incontinence and shock) and gastrointestinal tract (cramp, abdominal pain, vomiting and diarrhea) (2).

Mast cells interact also with innate and adaptive immune system in detection of harmful pathogens like viruses, bacteria, parasites (mainly helminths) and toxins (like Hymenoptera and snakes) (2). Mast cells participate also in wound healing, cancer and tumor progression, and diseases linked to increased fibrosis like idiopathic pulmonary fibrosis, multiple sclerosis (2,3).

Furthermore, clonal abnormal mast cell presenting KIT mutation (mainly D816V) are found in mastocytosis (4). The disease with broad symptomatology on the border of hematology, allergology, dermatology, endocrinology related to abnormal mast cell infiltration in the bone marrow, skin and other organs (5). As described above, the presence of the mast cell activation is found in many patients. The crucial part of diagnosis especially in case of anaphylaxis, is to identify patients with abnormal clonal mast cells who may suffer from systemic mastocytosis (SM) or primary Mast Cell Activation Syndrome (MCAS). The Departments of Allergology, Dermatology, Hematology of the Medical University of Gdańsk cooperates with the European Competence Network on Mastocytosis since 2005. We diagnose and treat more than 500 patients suffering from mastocytosis and MCAS. The following paper is based on the ECNM guidelines and our experience.
Mast Cell Activation Syndrome Criteria

According to Valent et al. algorithm, MCAS should be diagnosed when 3 criteria are met (2,6):

A) Typical and recurrent severe symptoms of excess mast cell activation (often diagnosed as anaphylaxis, affecting at least two organs). The symptoms typical to MCAS are: urticaria, flushing, pruritus, wheezing, angioedema, nasal congestion, tachycardia, hypotension, diarrhea. Headaches, memory loss and impaired concentration may also be present, however these symptoms are less specific.

B) Confirmed excess of mast cell activation in biochemical studies. Preferred marker is serum tryptase level elevation by 20% above upper limit of normal values or its by at least 20% above baseline plus 2 ng/ml after four hours post symptoms. Other metabolites include: serum and urine histamine and urine PGD$_2$, LTC$_4$, LTE$_4$ 11β-PGF$_{2α}$. Urine PGD$_2$ in 24-hour urine collection is considered the most specific marker of excess mast cell activation, though its availability is highly limited.

C) Positive response to symptom treatment as in mastocytosis. In consensus, this criterion should be fulfilled by antihistamine agents, however response to other drugs, such as leukotriene receptor blockers, systemic glucocorticosteroids, sodium cromoglicate may also be useful, though they are considered less specific, thus efficient in other than MCAS diseases. The withdrawal of symptoms should be complete or at least major in patients consideration (7).

In case of non-severe, transient symptoms (criterion A not fulfilled) and positive criteria B and C systemic or local (in case of limited range of skin symptoms) Mast Cell Activation (MCA) is diagnosed with similar to MCAS clinical approach (2). In other case, if patient does not respond to standard MCAS treatment and require recurrent epinephrine administration, MCA might be diagnosed provided that typical symptoms (A) and mediator elevation (B) are present and primary MCAS criteria are met (see below)(6).

After confirmed diagnosis, MCAS is categorized according to underlying cause. Primary MCAS is based on monoclonal mast cell proliferation, similar to SM, however not fulfilling its criteria. In this type CD25$^+$ mastocytes are observed in bone marrow biopsy and/or KIT D816V mutation. Diagnosis of mastocytosis is superior to MCAS, thus if at any point its criteria are fulfilled, MCAS is no longer considered.
Secondary MCAS is defined as mast cell activation due to comorbidities with negative D816V *KIT* and CD2/CD25 on bone marrow mast cells (8). The most typical cause is type 1 hypersensitivity according to Gell and Coombs classification that leads to persistent mast cell activation through allergen-specific IgE (9). *Hymenoptera* venom, food and drug intolerance and/or allergies are currently discussed as the most impactful on secondary MCAS. Though receptors for IgE (FcεRI) are considered the strongest mast cell activator, many different receptors are present on cell surface (10). Bacterial components might activate mast cell directly with Toll Like Receptors 2, 3, 4 and 6 and fMLP or through complement activation (11). Excess of hormones may also induce secondary MCAS through estrogen, progesterone, CRH and α-Melanocyte–stimulating hormone receptors. Persistent use of certain drugs such as opioids, muscle relaxants, intravenous contrast media, adenosine may also activate mast cells. In case of exclusion of primary and secondary causes, idiopathic MCAS may be diagnosed (9).

Importantly same patients may be diagnosed as having a primary and secondary MCAS such as in patients with mastocytosis and insect venom allergy who require specific immunotherapy (12,13). It is recommended in these group of patients to provide specific immunotherapy lifelong, in addition to antihistaminers’ treatment and the equipment of emergency kit including at least 2 autoinjectors of epinephrine (14).
When to diagnose patient towards MCAS?

The incidence of recurrent anaphylactic reactions is typical for patients with MCAS (15). If those reactions are associated with hypotension with cardiovascular collapse without skin changes like urticaria or angioedema the probability of MCAS diagnosis is increased even further (16,17). In case of acute symptoms, such as anaphylaxis, it is imperative to stabilize patient’s condition before start of any diagnostic process on etiology exploration.

MCAS investigation usually start from symptoms (anaphylaxis, pruritus etc.) and thus MCAS diagnostic algorithm should overlap anaphylaxis algorithm (see Figure 1). So far no diagnostic indications distinct from SM have been proposed for MCAS (2). However, in case of SM suspicion primary MCAS should always be considered. According to these algorithms MCAS should be suspected:

1) In all patients that have experienced anaphylaxis with hypotension with special consideration in patients with hymenoptera venom allergy and idiopathic anaphylaxis.
2) In case of severe osteopenia (T score<2) or osteoporosis in males and females before menopause
3) In patients with recurrent headaches and diarrhea especially on exclusion of all gastrointestinal tract diseases and food allergy/intolerance with no lesions in endoscopy.
4) In patients with unexplained pruritus and flushing. In this group cutaneous mastocytosis and chronic urticaria should be considered, though these may coexist with MCAS as well.
5) In case of unexplained neurological and psychiatric disorders with negative screening towards any neurological and endocrine disease.
6) In patients with confirmed mastocytosis in the skin (MIS).
7) In patients that experienced anaphylaxis with REMA score at least 2. The points are scored for male gender, lack of skin manifestation, fainting and high tryptase level. This score is generally used for bone marrow biopsy qualification (18).

Diagnostic algorithm was proposed initially by Valent et al. (2,19) and is presented on Figure 1. Bone marrow biopsy should be considered on diagnosis of MCAS.

Symptoms of the activation and release of mediators from mast cells (MCA) may result from the immune response or specific IgE independent mechanism of hypersensitivity (9). The
severity of MCA depends on the trigger, the type of reaction including sIgE-mediated mechanism and other chronic disorders.

Anaphylactic reactions occur in 0.05–2% of general population while in patients with mastocytosis more episodes have been observed—based on the reports from 22% to 49% in adults and between 6 and 9% in children (13,20). *Hymenoptera* stings considered a major cause of anaphylaxis in mastocytosis (4,13,20,21). The incidence of MCAS in patients with *Hymenoptera* Venom Allergy (HVA) ranged from 1 to 7.9% (12,20). In the majority of these patients anaphylactic reactions appear typically without skin involvement such as rash, blistering or angioedema but cardiovascular symptoms, such as hypotension and as a consequence loss of consciousness are predominantly observed. Therefore anaphylactic reactions without skin involvement could be a potential risk factor of MCAS (8,16). Patients with HVA with cardiovascular manifestation of anaphylaxis as well as patients with HVA and increased basal tryptase concentration in the serum should be diagnosed towards mastocytosis with bone marrow trepanobiopsy (12,20). It is suggested that these patients may have a very low MC burden so bone marrow examination should be performed in Reference Center of Excellence for Mastocytosis where appropriate, highly sensitive techniques are used (20).

The main procedures performed during diagnosing mast cell disorder must include: dermatological examination, basal serum tryptase level measurement and the presence of *KIT* gene mutations, notably D816V analysis.

### How to exclude mastocytosis in the skin? When do the skin biopsy?

The term mastocytosis in the skin (MIS) refers to heterogeneous skin lesions which are typical for various forms of cutaneous mastocytosis (CM) (7). It is a provisional diagnosis which can be used until SM-related criteria are checked and final diagnosis of CM or SM is established. CM by definition presents with lack of internal organs involvement (7,22). Adults suffer from SM with or without skin involvement (19,22,23). The most common clinical presentation of MIS in adults is a maculopapular form (MPCM), previously termed *urticaria pigmentosa* (22,23). It has been estimated that approximately 95% of patients with ISM present with MPCM, whereas around 50% of patients with advanced forms of SM exhibit skin lesions (19,22,24). That is why MIS is considered a significant diagnostic indicator of mastocytosis. To exclude MIS all patients with mast cell mediator-related
symptoms have to be examined by dermatologists and undergo a skin biopsy if CM is suspected.

MPCM is characterized by small, round, brown or red monomorphic lesions that intensify usually upon rubbing, exposure to heat or emotional stress (22). Mechanical irritation of CM manifestations may provoke skin mast cell degranulation with redness and urticaria on the skin surface. This reaction called Darier’s sign is highly specific to CM (22,25). In contrast to the Darier’s sign, dermographism is elicited by stroking a nonlesional skin. Small monomorphic lesions correspond to a monomorphic variant of MPCM which is the most typical for adulthood-onset mastocytosis. Less frequently adult patients exhibit brown or red larger lesions of different size and shape typical for a polymorphic variant of MPCM (22). Skin lesions may vary in terms of their numbers, shape, elevation and pigmentation. Patients may have only a few lesions, usually localized on the thigh and trunk. Skin lesions may be visible also at other body sites as disseminated macules, papules, plaques or nodules. In some patients skin lesions tend to show confluence or they are accompanied by telangiectasias (22). Rarely, adult patients suffer from diffuse cutaneous mastocytosis (DCM), the most severe form of CM, due to mast cell infiltration involving almost the entire skin (26,27). DCM usually occurs at birth or in early infancy and presents with generalized erythema and blistering. In adults, a generalized thickening of the skin with the leather-grain appearance and the pronounced Darier’s sign are prominent features of DCM (26). Patients with MIS may experience mast cell mediator-related symptoms, both systemic and skin-specific such as flushing and pruritus (blistering occurs only in children) (7,22).

Diagnosis of MIS is established by inspecting the skin and performing a skin biopsy (22). Numbers of mast cells are increased 4- to 8-fold in the lesional skin of CM patients (around 40 mast cells/mm²) (7,22,28). It is recommended to use an antibody against tryptase as a standard immunohistochemical marker or anti-KIT (CD117) to visualize skin infiltrate by mast cells (5,18,24). In unclear cases, if the histology is not diagnostic, the presence of D816V KIT mutation at codon 816 in lesional skin confirms the diagnosis of MIS (7,22).

**Tryptase level and other mediators**

The crucial, first line examination in patients with suspected mastocytosis or primary MCAS is tryptase level in peripheral blood (19). In the lack of urticaria pigmentosa, the level below 15 ng/ml and no increase during the suspected reaction should be followed up. The tryptase
level above 25 ng/ml is an indication for the bone marrow studies including histopathology, cytology, flow cytometry and detection of the \textit{KIT} mutation (19). Cases with the level between 15 and 25 ng/ml presenting REMA score $\geq2$ or D816V mutation detected in peripheral blood should also undergone bone marrow studies (18).

The elevated tryptase level may be related to other comorbidities like hematologic, non-hematologic reactive and other etiologies (2). Hematologic diseases include chronic leukemia (myeloid, eosinophilic, basophilic), acute basophilic or myeloid leukemia, myelodysplastic syndrome, myeloproliferative neoplasm especially with mutated PDGFR or FGFR, myelomastocytic leukemia (2,29). Non-hematologic reactive conditions with elevated tryptase level are allergic disorders mainly exacerbated, chronic urticaria, chronic inflammatory diseases and chronic helminth infection. Other disease include end stage kidney disease, familial hyper alpha-tryptasemia. It can be found rarely in healthy individual or as a false positive result due to heterophilic antibodies (2). Additional mediators such as histamine in plasma or urine, histamine metabolites in urine or prostaglandin metabolites in 24 – hour urine, may also be used as indicators of MCA (30). The positive result should be based on event related increase of at least two of these mediators or, preferably, at least 50% higher values after the reaction in comparison to the baseline result (2).

\textbf{The importance of gene studies in MCAS}

From three MCAS variants only primary variant of disease have clonal somatic genetic aberration. No specific mutations have been found in patients with secondary or idiopathic MCAS.

The crucial element in pathogenesis of the primary MCAS disease is the presence – somatic activating D816V \textit{KIT} mutation in exon 17 in mast cells detected in peripheral blood or bone marrow (2,6,31).

This mutation is observed in $>80\%$ adults cases with SM. In pediatric patients, mainly with cutaneous mastocytosis (CM), 25\% cases have no mutation, 35\% have \textit{KIT} D816V (D816I or D816Y) mutation and 40\% have other mutations coding extracellular part of SCF receptor (32).
Additional genetic changes observed in mastocytosis patients

The presence of activating \( KIT \) mutations are not the only factor determining variety of clinical manifestations of mast cell disorders. Other mutations or gene polymorphisms are vital for regulation of mast cell proliferation or activation and influence final clinical outcome (33).

The severity of anaphylactic reactions might be increased by activated cascade of intracellular tyrosine kinases: Kit, Lyn, Syk and Fyn in pathological mast cells (4,34). On the other hand, the presence of D816V \( KIT \) gene mutations do not change significantly the course of anaphylactic reactions.

Recently, research on the association of polymorphism of genes and clinical outcome of the disease have been conducted (35,36). Clinical manifestations of mastocytosis are influenced by polymorphisms of interleukin-13 promoter gene (1112C/T) and interleukin 4 receptor α-chain (Q576R). Polymorphism of – 1112C/T IL-13 gene increase risk of developing systemic mastocytosis. The study of Lange et al. revealed a potential role of polymorphic variants of IL-31 gene in the pathogenesis of mastocytosis (37). It was shown that IL-31 IVS2+12AA genotype and IVS2+12A allele appeared far more frequently in patients with mastocytosis compared to control subjects increasing the risk of SM development. Moreover, the presence of -2057AA genotype increased risk of SM in adults (37).

The study of Rausz et al. which analyzed IL6R Asp358Ala polymorphism showed that carriers of the AA genotype had a 2.5-fold lower risk for mastocytosis than those with the AC or CC genotypes (\( OR=0.40 \ CI 0.2 0.8 \ p=0.008 \) (33).

In the of study of Górńska et al. increased expression the TRAF4 gene in mastocytosis patients with food hypersensitivity and decreased expression of B3GAT1 gene in mastocytosis patients with IVA have been found (38). The study of Niedoszytko et al. on whole genome expression showed that the genes identified in pathways leading to cancer development take part in the risk of an anaphylaxis on exposure to insect venom. Patients who did not respond to insect sting presented more abnormalities in gene expression typical for neoplastic diseases (39). Higher expression of TRAF4 due to Th2 allergic inflammation is in line with another observation highlighting the role of IL-13 gene polymorphism in the pathogenesis of mastocytosis and frequent food-related hypersensitivity reactions in this group of patients (35).
Mutations in epigenetic regulators genes in mastocytosis

Abnormalities in regulation of epigenetic mechanisms of gene expression may affect pathogenesis of mastocytosis through: specific micro RNA expression, loss of suppressor gene function, activation specific oncogenes (tyrosine kinases, signal transduction proteins), incorrect of replication and DNA repair processes, apoptosis, and by causing instability of mast cell genome (40–42). Aberrant expression of micro RNAs is detected in mast cells with *KIT* mutation. Lee et al. indicated that cells with *KIT* mutations have lower expression of miR -539 and miR 381. This miRNAs are involved in inhibition of expression of MITF (Microphthalmia-associated transcription factor), a regulator of mast cells and melanocyte development, and melanin and tryptase synthesis – **Figure 2** (40–42)

The recent studies using sequencing of a multiple genes in search for typical mutations observed in myeloid malignancies have also found a list of somatic mutations frequently observed in patients with mastocytosis. (43–47) These included genes encoding factors regulating splicing process, signaling transmission and epigenetic regulations. Mutations in genes encodes factors implicated in epigenetic process are frequently observed in clinically advanced form of mastocytosis and are associated with poor prognosis and life shortening. The most frequently mutated genes are: TET-2 (demetylations of DNA ), DNMT3A (metylations of CpG islands ), ASXL1 (chromatin silencing and remodeling) and IDH2 (regulation of histone methylation) – **Figure 2** (43–47).

Management of patients with MCAS and HVA.

The aim of chronic treatment is to avoid symptoms by prophylactic use of antimediaters’ drugs (5). Primary choice are histamine receptor blockers (HR1 and HR2). Patients with gastrointestinal tract symptoms may benefit from addition of proton pump inhibitors. Some patients also benefit from administration of cromones, low dose of glikokorticosteroids and in case of psychological disorders – antidepressants (5,48–50).

It is important to prevent any future allergic or hypersensitivity reactions based on correct diagnosis and management, including the equipment of self-injector of epinephrine and specific venom immunotherapy in confirmed venom allergy. According to the guidelines all patients with anaphylaxis after insect sting in medical history should be diagnosed towards
insect venom allergy (IVA) and qualified to immunotherapy (51). Diagnosis of IVA is confirmed based on the symptoms of anaphylaxis as well as an identification of the stinging insect and confirmation of the specific IgE-mechanism of the systemic reaction. According to the recommendations skin tests and the detection of serum specific IgE to insect venoms should be performed at least 2 weeks after the post-stinging reaction (52). If the diagnosis is not confirmed, the same procedures should be repeated in a few weeks’ time. In these patients higher risk of MCAS is found (53). On the other hand in patients with mastocytosis and insect venom anaphylaxis history the correct diagnosis is hard to confirm based on standard procedures (54). Negative skin tests results and not detectable sIgE may be observed in these cases presumably because of the absorption of sIgE on the surface of mast cells (13). In these cases sIgE detection against recombinant allergens or molecular diagnostic approaches such as basophil activation test may improve the diagnosis accuracy and enable the IVA recognition.

There is no difference in the recommended therapy in patients with IVA regardless of the presence of MCAS. Besides antihistaminic treatment all patients without contraindications are qualified to immunotherapy and should be equipped with emergency kit including epinephrine autoinjector. Importantly, venom immunotherapy as the only disease modifying treatment should be performed lifelong in patients with mastocytosis or MCAS according to the current guidelines. Considering the analysis of large database there is suggestion to perform the induction of VIT with modified – less aggressive build-up phase protocol due to some side effects, although their number didn’t achieve statistical significance, observed during rush-modified course (13). Besides that no adverse reaction was observed during the maintenance treatment and no discontinuance regarding side effects related to insect venom immunotherapy was reported (55). The frequency of adverse events in MCAS patients is approximately 18,9%, even though they are reported from 0% to 46% and is similar to the general population (12,13). However, in patients with mast cell disorders the risk of anaphylaxis is sustained and the possibility of systemic reaction after being stung by an insect whose venom was not used for VIT may occur (56).

Therefore all patients with mast cell disorder should be equipped with emergency kit including self-injector of epinephrine even though the maintenance phase of venom immunotherapy has been performed (56).


Mastocytosis; the American Academy of Allergy, Asthma & Immunology; and the European Academy of Allergology and Clinical Immunology. J Allergy Clin Immunol. 2016 Jan;137(1):35–45.


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Figure 1 MCAS diagnostic algorithm. REMA - Red Española de Mastocitosis; MIS – Mastocytosis In the Skin; MCAS – Mast Cell Activation Syndrome; IA – Idiopathic Anaphylaxis; EIA – Exercised Induced Anaphylaxis; FDEIA – Food Dependent Exercised Induced Anaphylaxis; SM – Systemic Mastocytosis
**Figure 2** Main genetic mutations and epigenetic changes observed in mastocytosis.