

Survey of Mast Cell Mediator Levels from Patients Presenting with Symptoms of Mast Cell Activation

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Keywords

Leukotrienes C_4 and E_4 · Mast cell activation · Prostaglandin D_2 · N-methylhistamine · Tryptase

Abstract

Introduction: Although 4 mast cell mediators can be routinely measured, the results of initial testing to evaluate symptoms of mast cell activation have not been widely reported. **Objective:** We examined the results of mast cell mediator tests used to assess patients with mast cell activation symptoms during a 5-year time span. **Methods:** After excluding patients with alternative diagnoses, records of 108 patients were reviewed for initial mediator test results. Mediators included serum tryptase plus urinary N-methyl histamine (N-MH), leukotriene (LT) E_4 , and 11 β -prostaglandin (PG) $F_{2\alpha}$ or 2,3-dinor-11 β -PGF $_{2\alpha}$ (BPG). **Results:** Most commonly, either a single measured elevation of 1 mediator (48.1%) or elevations of 2 (33.3%) mediators was found at baseline, during symptoms or at both time points. Elevated levels of a single mediator in order of frequency were: BPG > tryptase > LTE $_4$ > N-MH, and for two mediators: BPG + tryptase ($n = 16$ cases) > BPG + LTE $_4$ ($n = 9$) > BPG + N-MH ($n = 6$). Elevations in 3 mediators ($n = 8$) or 4 mediators ($n = 2$) were much less frequent. Monoclonal mast cell activation syndrome ($n = 6$),

and systemic and cutaneous mastocytosis ($n = 4$) were also infrequent. Baseline plus symptom-associated tryptase values were obtained in only 7 patients. **Conclusions:** This survey suggests that elevations of 1 or 2 mediators are the most common (total 81.4% of cases) findings from initial tests for mast cell activation. Elevated levels of BPG were most commonly found both singly and in combination with other mediators, followed by the finding of elevated levels of tryptase. Baseline plus symptom-associated tryptase levels were measured in only a minority of patients.

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Introduction

It is not overstating the fact that during the past several years mast cell activation syndrome (MCAS) has taken the allergy world by storm. “Mast cell degranulation” was first mentioned in association with mastocytosis in the literature nearly 60 years ago [1], and the recognition of “mast cell activation” (MCA) associated with clinical symptoms first appeared nearly 40 years ago [2].

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In many respects the possibility of MCAS has become an unruly compendium for clinical symptoms and complaints that physicians are frequently at a loss to explain, and reliance on the finding of increased tryptase and other mast cell (MC) mediators plays an important role in diagnosing MCAS. Despite the fact that a consensus proposal for defining MCAS has been published and widely accepted [3], these guidelines are commonly ignored, and in real-world clinical practice a diagnosis of MCAS has been used to rationalize otherwise unexplainable localized or systemic symptoms.

The consensus proposal requires 3 components to confirm a diagnosis of MCAS: (1) symptoms that are commonly associated with release of MC mediators, affecting 2 or more organ systems, (2) documented symptom-associated increase of the level of serum tryptase by $20\% + 2 \text{ ng/mL}$ above baseline levels, and (3) relief of symptoms by the use of medications known to prevent synthesis or release of MC mediators or that block their receptors [3]. Unfortunately, only single aspects of this consensus definition, such as symptoms of MCA, are commonly used to justify this diagnosis for individual cases.

In addition, symptoms can be difficult to reconcile with the known effects of mediators released during MC degranulation. "Acceptable" complaints for MCAS vary from publication to publication [4–6], may diverge substantially, and have included chronic inflammatory disorders, neurologic and psychiatric symptoms, as well as more "traditional" symptoms associated with MC mediators. Clinical presentation may also vary, with some patients complaining of chronic day-to-day symptoms and some patients who have periods of normality punctuated by acute attacks.

Patients have been (mis)diagnosed with MCAS because they experience subjective improvement of their symptoms with antihistamines, montelukast, or cromolyn sodium, although these medications can provide relief in many disorders. One possible explanation of such subjective benefit is the recognized placebo effect reported when treating allergic disorders and related clinical conditions, such as "multiple chemical sensitivities" [7–10].

The finding of a substantial, symptom-associated rise in tryptase, though helpful to signal MCA, does not aid the clinician to understand which mediators are increased and contributing to symptoms. MC produce many products (histamine, leukotriene $[LT]C_4$, prostaglandin $[PG]D_2$), and if urinary levels of the metabolites of these mediators have not been measured, treatment proceeds in a

blinded fashion, often with poor results. Although current consensus criteria for MCAS allow the use of other MC mediators to confirm the diagnosis, the minimal diagnostic increase for each has not been established. Currently, these non-tryptase mediators are not a component of defining MCAS.

Lastly, the difficulty of diagnosing or of excluding MCAS is further complicated by several obstacles. These include: (1) the difficulty of actually obtaining symptom-associated samples in "real world" practice. Time and again patients who seek emergency care for symptoms are ignored when they encounter EMT personnel or a busy emergency room physician unfamiliar with MCAS who refuses for various reasons to obtain the necessary samples; (2) the failure to evaluate for the possibility of more common endocrine, neurologic, psychiatric, and cardiovascular disorders, that could give common symptoms; (3) the discovery of new disorders associated with elevated tryptase levels such as the recently described familial hypertryptasemia syndrome due to an increased *TPSAB1* copy number with many, but not all, of these patients having symptoms consistent with MCA [11]; and (4) the failure to recognize the occurrence of elevated tryptase levels that are found in other conditions such as myeloid neoplasms [12].

Several excellent reviews of systemic mastocytosis (SM) and MCAS have recently been published, including guidelines to aid in the diagnosis and treatment of these disorders [13, 14]. However, as patient symptoms are generally the initial point of their contact with physicians, the present 5-year retrospective survey was performed to examine corresponding MC mediator levels for patients with symptoms of MCA.

Methods

We utilized Mayo Clinic databases to search for patients aged 18 years and older with a diagnosis of MCAS seen during a 5-year interval (January 10, 2012 to January 10, 2017). We verified presenting symptoms and MC mediator levels (baseline, event-associated, or both). "Baseline" as used in this report relates to a period of time that was not associated with acute symptoms.

Laboratory Tests

The normal value for serum tryptase (ImmunoCAP Tryptase; Phadia AB, Uppsala, Sweden) was $<11.5 \text{ ng/mL}$. Urinary N-methyl histamine (N-MH) and LTE_4 were measured by liquid chromatography-tandem mass spectroscopy (LC-MS/MS). Normal ranges for urinary mediator levels were: N-MH ($30\text{--}200 \mu\text{g/g creatinine [Cr]}$) and LTE_4 ($<104 \text{ pg/mg Cr}$). For PGD_2 metabolites, during the years surveyed, 3 different assays were utilized: 24-h total excretion of $11\beta\text{-PGF}2\alpha$ (normal $<1,000 \text{ ng/24 h}$; ELISA assay, Cay-

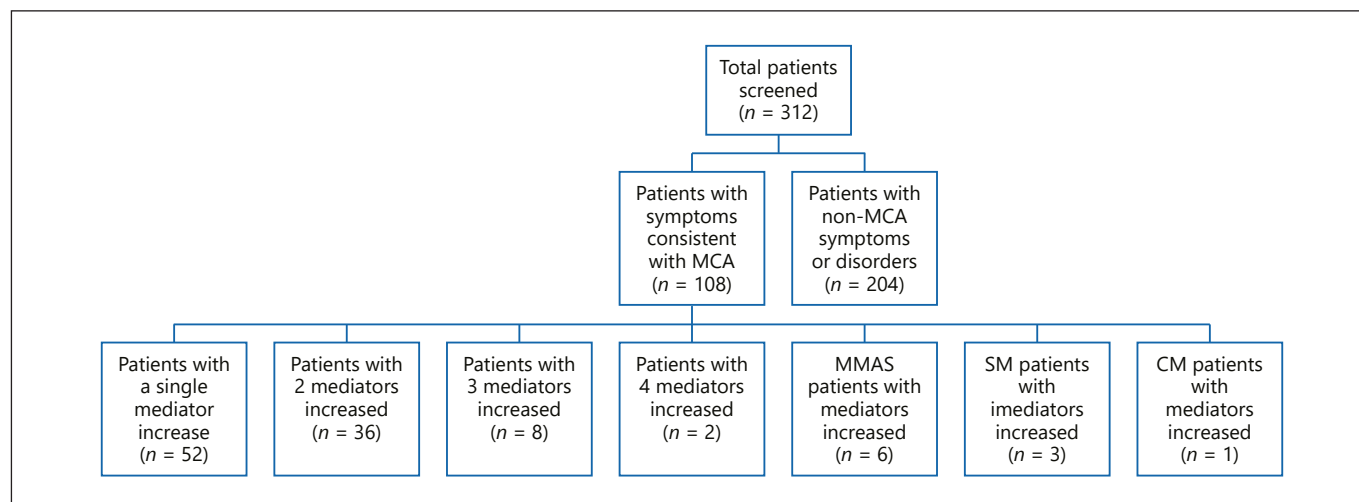


Fig. 1. Summary of cases reviewed.

man Chemicals) and subsequently 2 assays with differing normal ranges for 2,3-dinor-11 β -PGF₂ α expressed as pg/mg Cr (LC-MS/MS). For uniformity the values were expressed as a ratio of “patient value/maximum normal value” for the assay used at that time (“normalized”); the abbreviation for beta prostaglandin, “BPG,” is utilized throughout for all of these PGD₂ metabolites. At our clinic the assay for LTE₄ became a routine clinical assay in March 2014 and therefore results are not available for patients reviewed prior to that date. All assays were performed at Mayo Medical Laboratories. Standard collection for urine is a 24-h specimen. Since mid-2016 physicians have had the option of ordering of the urine collection on random “spot” samples as all the urine mediator results are now given as “per g or mg Cr.”

After excluding patients with symptoms not related to MC activation and with other disorders (see below), we examined the remaining patients including those with monoclonal mast cell activation syndrome (MMAS), SM, and cutaneous mastocytosis (CM) for available MC mediator assays that had been obtained at baseline and/or were associated with acute symptoms. Examples of excluded diagnoses included postural orthostatic tachycardia syndrome (POTS), somatic symptom disorder, immune deficiencies, dysautonomia, neurogenic disorders, chronic fatigue syndrome, Ehlers Danlos syndrome, seasonal allergies, myofascial pain, fibromyalgia, and visceral hypersensitivity. Patient use of aspirin (ASA) and/or nonsteroidal anti-inflammatory drugs (NSAIDs) is indicated.

Results

A total of 312 patients were identified who had MCAS recorded as one of their clinical diagnoses. These charts were then individually reviewed for the presence of symptoms consistent with MCA; 204 patients were rejected, leaving 108 patients with symptoms consistent with MCA

and who also had baseline, event-related, and/or baseline plus event-related MC mediator assays (Fig. 1). The number of patients using aspirin and/or an NSAID is also included. No patient was using zileuton. The total number of assays for each mediator and the percent of elevated mediator for each assay and for each patient group are given in Table 1.

Patients with a Single Mediator Elevation

A total of 52 patients (48.5%) had the finding of a single mediator elevation either in assays at baseline (46 patients) or associated with symptoms (6 patients). The mediator most commonly elevated was BPG, increased in 58% of assays, followed by tryptase, increased in 30% of assays. LTE₄ (27.3%) was increased nearly as frequently as tryptase, while N-MH (6.1%) was elevated much less commonly (Table 1). The most common symptom for the group overall was flushing. The baseline values ($\pm$ standard deviations) for the individual mediators were tryptase: 20.6 ± 9.5 ng/mL; LTE₄: 277.3 ± 150.5 pg/mg Cr; N-MH: 209.5 ± 12 μ g/g Cr; and for “normalized” BPG, with individual results expressed as a ratio of “patient value/maximal normal value” for the assay used: 1.9 ± 0.9 . For this group as a whole, 8 patients were taking aspirin and 3 patients were using an NSAID on an “if needed” basis.

During acute symptoms, BPG was the mediator most commonly increased. Among patients who had a single mediator elevation measured, 2 patients had both baseline and symptom-associated tryptase levels obtained. These patients had the required increase of $20\% + 2$ ng/

Table 1. Mediator assay numbers by groups listed, percent elevated levels by assay, and number of patients per group

Mediator	Total assays performed for the mediator, <i>n</i>	Patients with baseline and/or acute increase in the mediator, <i>n</i>	Assays showing increased level, %	Patients with increase in the mediator, %
<i>Patients with 1 mediator increase (n = 52)</i>				
Tryptase	51	15	29.4	28.8
N-MH	52	2	3.9	3.9
LTE ₄	23	6	26.1	11.5
BPG	51	29	56.9	55.8
<i>Patients with 2 mediators increased (n = 36)</i>				
Tryptase	37	21	56.8	58.3
N-MH	38	8	21.1	22.2
LTE ₄	21	13	61.9	36.1
BPG	36	32	88.9	88.9
<i>Patients with 3 mediators increased (n = 8)</i>				
Tryptase	10	6	60	75
N-MH	10	5	50	62.5
LTE ₄	8	8	100	100
BPG	10	7	70	87.5
<i>Patients with 4 mediators increased (n = 2)</i>				
Tryptase	2	2	100	100
N-MH	2	2	100	100
LTE ₄	2	2	100	100
BPG	2	2	100	100
<i>MMAS (n = 6)</i>				
Tryptase	7	6	85.7	100
N-MH	7	3	42.9	50
LTE ₄	2	1	50	16.7
BPG	7	6	85.7	100
<i>CM and SM (n = 4)</i>				
Tryptase	5	3	60	75
N-MH	5	3	60	75
LTE ₄	2	1	50	25
BPG	5	3	60	75

Patient groups by number of increased mediators or by diagnosis (in parentheses) = number of patients in the group.

mL, which therefore qualified as an MC activation event according to current criteria. These 2 patients represent 3.9% of the patients who had single mediator elevations (Table 2).

Patients with 2 Mediator Elevations

A total of 36 patients (33.3%) had laboratory assays demonstrating increased levels of 2 mediators either at baseline (33 patients) or during symptoms (3 patients). The 3 most frequently increased baseline mediator “pairs” in this group were: (1) BPG + tryptase (14 patients); (2) BPG + LTE₄ (9 patients); and (3) BPG + N-MH (6 pa-

tients). The most common symptom for each of these 3 patient groups were, respectively: pruritus, flushing/urticarial/angioedema (equal), and flushing. Table 1 lists the total number of assays for each mediator and the number and percentage of increases found.

Three patients had 2 mediators elevated at the time of symptoms. Both baseline and “20% + 2 ng/mL” increased symptom-related tryptase levels were obtained in 2 of these patients. In one patient there was an accompanying increase of BPG excretion and in the second patient an accompanying increased level of LTE₄ (no baseline value obtained) (Table 2). Therefore, in this entire group with increased levels of 2 mediators, only 2 patients (5.6%) had increases of tryptase that satisfied the criteria for MCAS events. For this group as a whole, 9 patients were taking aspirin and 2 were taking an NSAID.

Patients with 3 or 4 Mediator Elevations

The finding of increases in 3 or 4 mediators was much less common (Table 1). Elevated levels of 3 mediators were found in a total of 8 patients (7.4%), with baseline increases in 6 and symptom-associated increases in 2 patients. For the 4 patients with increased levels of tryptase + LTE₄ + BPG, the most common symptom was pruritus. In 2 patients with baseline increases in N-MH + LTE₄ + BPG, the most common symptom was flushing. One patient was found to have a >20% + 2 ng/mL increase in tryptase during symptoms that was accompanied by increases in N-MH and LTE₄ (Table 2). A total of 5 patients were taking aspirin.

Two patients had elevated levels of all 4 mediators (1.9%) (Table 1). One patient had baseline elevations, but no additional symptom-associated samples were obtained. The second patient, taking aspirin 81 mg/day, had baseline and acute measurements. The baseline levels for this patient were all normal save for LTE₄ (125 pg/mg Cr). During symptoms all mediators were elevated with markedly increased levels of LTE₄ and BPG (9.4- and 12.4-fold increase above baseline values, respectively) while tryptase and N-MH increases were more modest.

Patients with MMAS

Six patients, (5.6%) of the total population, were diagnosed with MMAS. All of the patients who had baseline measurements showed increased levels of tryptase and BPG. Additionally, N-MH was elevated in 3 patients and LTE₄ in 1 patient.

One MMAS patient had both baseline and symptom-associated measurements. This patient had a normal baseline tryptase value of 8.7 ng/mL which increased sub-

Table 2. Baseline and symptom-related tryptase levels meeting the “20% + 2 ng/mL” requirement including any associated urinary MC mediator levels obtained

	Tryptase-baseline ¹	Tryptase-sx ¹	N-MH-baseline ²	N-MH-sx ²	LTE ₄ -baseline ³	LTE ₄ -sx ³	BPG-baseline ⁴	BPG-sx ⁴
<i>Single MC mediator increase</i>								
Pt 1	13.1	29.7	nd	nd	nd	nd	nd	nd
Pt 2	3.5	24.1	87	nd	nd	nd	0.988	nd
<i>Two mediators increased</i>								
Pt 1	3.6	27.1	88	180	nd	nd	1.204	13.25
Pt 2	4.6	26.2	81	81	nd	357	0.721	0.52
<i>Three mediators increased</i>								
Pt 1	5.2	28.4	5,025	25,840	247	1,921	0.267	0.279
<i>Four mediators increased</i>								
None with baseline + acute tryptase								
<i>MMAS</i>								
Pt 1	8.7	52.9	155	219	nd	nd	0.31	2.09
<i>SM</i>								
Pt 1	6.13	12.3	91	117	nd	nd	0.614	5.79

MC, mast cell; sx, symptom; Pt, patient. ¹ Reference range <11.5 ng/mL. ² Reference range 30–200 µg/g Cr. ³ Reference range <104 pg/mg Cr. ⁴ BPG expressed as ratio of “patient value/maximal normal value” (no increase = “1.0”). nd, mediator not assayed.

stantially during symptoms (52.9 ng/mL). Baseline levels of N-MH and BPG were normal, but all 3 mediators (excepting LTE₄ which was not measured) increased acutely. Hence, 1 of the 6 MMAS patients actually had the required increase in tryptase to qualify as an activation event (Table 2). In this group, 2 patients were using aspirin or an NSAID. The most common symptoms among MMAS cases were loss of consciousness (4 patients), flushing (3 patients), and tachycardia (3 patients).

Cutaneous and Systemic Mastocytosis

A total of 4 cases (3.7%) of CM (1 patient) or SM (3 patients) were identified.

One patient with CM and negative bone marrow examination had baseline increased BPG and normal tryptase and N-MH values (LTE₄ not measured) while taking aspirin 162 mg b.i.d.

Two patients with SM had mediator measurements only in baseline state. Both showed increased levels of tryptase and N-MH. One patient additionally had an increased excretion of BPG while the second patient, who was taking aspirin, had normal BPG excretion and increased LTE₄ excretion.

One additional SM patient had both baseline and symptom-associated measurements of 3 mediators that

showed normal baseline levels of N-MH, BPG, and tryptase. During symptoms N-MH did not increase while both BPG (0.614 → 5.787 [normalized values]) and tryptase (6.13 → 12.3 ng/mL) increased substantially, with tryptase increasing >20% + 2 ng/mL. LTE₄ was not measured in this patient.

A summary of all patients having both baseline and symptom-associated tryptase levels is presented in Table 2. Where obtained, additional values for the other MC mediators are included.

Discussion

The results of this survey illustrate that when patients with symptoms of MCA are initially evaluated, a limited number of MC mediators with raised levels can be expected. Although tryptase was commonly elevated, increased urinary excretion of BPG was even more common. Single mediator increases were found most commonly (52 of 108 cases, 48%), mainly in baseline conditions with only sporadic samples obtained during acute symptoms.

In order of frequency, an increased BPG level was followed by increased tryptase > LTE₄ > N-MH. The fre-

quency of increased LTE_4 may be underrepresented because this assay was available for only a portion of the sample accrual dates due to its introduction in 2016. The importance of measuring all 4 mediators is reflected in the fact that omitting assays for 2 of the mediators, N-MH and LTE_4 , would have resulted in missing 17% of patients with a single mediator increase. There were only sporadic samples obtained during symptoms, thereby making any statement of what constituted a significant increase of the non-tryptase mediators problematic.

However, for patients who had both baseline and symptom-related single mediator measurements the most commonly increased mediator, again, was BPG (3 cases) followed by tryptase (2 cases) and N-MH (1 case).

Reported increased levels of more than one MC mediator in MC activation are rare [15, 16], with more emphasis frequently placed on symptoms, differential diagnosis, and treatment [17, 18]. In the current series overall, 36 of 108 patients (33 baseline plus 3 during symptoms) were found to have increased levels of 2 mediators. BPG again figured frequently in mediator pairs that were increased and was associated with an increase in tryptase in 16 patients (the most frequently increased pair), with a simultaneous increase of LTE_4 in 9 patients and of N-MH in 6. In comparison, excluding its association with BPG an increased tryptase was associated with "another" mediator in 4 cases (LTE_4 in 3, and N-MH in 1). Hence, elevations of a normalized BPG value plus "another" mediator elevation occurred in 32 of 36 patients (88.9%) versus 21 of 37 patients (56.8%) with an elevated tryptase plus "another" mediator elevation. These findings again point out the importance of quantitating multiple MC mediators, as tryptase assays alone would give an incomplete picture of what biochemically occurred.

In a previous study, measurement of 24-h urinary $11\beta\text{-PGF}_2\alpha$ in addition to serum tryptase improved identification of MCAS patients. In that report elevated excretion of $11\beta\text{-PGF}_2\alpha$ was found more commonly (17 of 25 patients) than increased serum tryptase (10 of 25) or N-MH (2 of 25). The low number of patients with increased N-MH represented 8% of the cases reported, similar to the 3.9% reported in the current series. Nine patients with elevated urinary $11\beta\text{-PGF}_2\alpha$ had a symptomatic response to aspirin administration (dose 81 mg/day to 500 mg b.i.d.) and 8 of the 9 had a lower excretion of this mediator while taking aspirin (re-measurement not completed in 1 patient) [15].

The number of patients who had 3 (8 patients) or all 4 (2 patients) mediators increased was quite small. While

the small number of cases in these groups makes it difficult to draw any firm conclusions, mediator increases above baseline could be substantial. In one patient with symptom-associated increases in 3 mediators, tryptase increased 5.7 times the baseline level while for N-MH the increase was 5.1-fold and for LTE_4 7.8-fold (BPG did not increase). Even more impressive were the symptom-associated increases above baseline levels for one patient with increases in all 4 mediators. In this case N-MH increased a modest 0.2 times the baseline level, while for tryptase the increase was 5.6-fold, LTE_4 9.4-fold, and BPG 12.4-fold.

Patients with MMAS were also a small proportion of the overall population (5.6%). Increased tryptase and BPG levels were found in all of these patients, either at baseline or associated with symptoms

Cutaneous and systemic mastocytosis comprised a total of 4 cases (3.7%). For the patient with CM, only BPG was elevated. For the 2 SM patients monitored at baseline, both had increases in tryptase and N-MH, while one each had an increase in BPG or LTE_4 . The third SM patient had symptom-associated increases in tryptase and BPG. In 2 of the SM cases bone marrow biopsy showed <5% involvement, while in the third the bone marrow biopsy (obtained elsewhere) report did not list a percentage. From their clinical histories all were indolent SM.

Although tryptase has remained the gold standard of diagnosing MCAS, this survey shows that measurements of other MC mediators, particularly BPG, are commonly elevated in patients with symptoms of MCA. Paired baseline and symptom-associated tryptase levels were obtained in only a fraction of the cases reviewed. Increases in plasma or urinary histamine metabolites, PGD_2 metabolites, or LTE_4 at least 50% from baseline have been suggested as providing an indication of MCA [13]. A doubling above baseline has also been proposed [13]; however, there is no agreed-on value. In a large number of our patients "normalized" BPG was found to be high as a single measurement or along with other mediators either at baseline (70 patients) or during symptoms (9 patients). In comparison, tryptase elevations were found in 43 patients at baseline and 10 patients during symptoms

PGD_2 is not produced by human basophils [19] and is only produced in tiny amounts by murine basophils [20]. Overproduction of PGD_2 has been reported in conditions related to MCA such as human anaphylaxis [21]. Although other cell types have been reported to produce PGD_2 , the clinical conditions in which this occurs are narrow and distinct from MCAS. For example, in aspirin-

exacerbated respiratory disease (AERD), a condition in which circulating and tissue eosinophils are increased, eosinophils as well as MC can be a source of PGD₂ [22]. Eosinophilia, however, is not a diagnostic feature of MCAS. Although PGD₂ can be produced by TH₂ cell lines in vitro [23], the amount (~2–6 ng/10⁶ cells) was less than half of the lowest amount of PGD₂ produced in vitro by partially (70%) purified human MC (12–36.2 ng/10⁶ cells) [24]. No increase in the number of TH₂ cells has been reported in MCAS. Dendritic cells are also a source of PGD₂, but the amount secreted is less than 1% of the amount secreted by MCs and 20-fold less than the amount secreted by TH₂ cells [25].

There are weaknesses to a review of this type. This was a retrospective survey in which potential MCAS cases were screened based on presenting symptoms. Accepted symptoms were those commonly acknowledged to be due to MC mediator release as described in the literature [3]. In this survey patients with a diagnosis of primary inflammatory and neurologic disorders such as fibromyalgia, POTS, dysautonomia, neurogenic pain syndrome, and others for which there is more limited acceptance of being due to MCA were rejected from inclusion. There was also limited follow-up available for the majority of patients surveyed. In addition, the assay for LTE₄ was not available for the entire time of the study.

In summary, this review offers a glimpse of the results of mediator levels used to investigate patients with symptoms of MC activation. Of interest, and supported by previous reports in the literature, was the finding of the frequent elevation of BPG levels in both baseline states and associated with symptoms, an occurrence that exceeded that of elevated serum tryptase values.

Statement of Ethics

The study protocol was approved by the Institutional Review Board of the Mayo Clinic. There was no direct patient contact, evaluation, or treatment and patients were not required to give written informed consent.

Disclosure Statement

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