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Learned cautions regarding antibody testing in mast cell activation syndrome

https://doi.org/10.1515/dx-2023-0032
Received April 20, 2023; accepted July 26, 2023;
published online August 14, 2023

Abstract

Objectives: To describe patterns observed in antibody titer trendlines in patients with mast cell activation syndrome (MCAS, a prevalent but underrecognized chronic multisystem inflammatory disorder of great clinical heterogeneity) and offer clinical lessons learned from such pattern recognition.
Methods: The available records of 104 MCAS patients drawn from the authors' practices were reviewed, including all antibody tests therein.
Results: All patients had positive/elevated antibodies of various sorts at various points, but for most of the antibodies which were found to be positive at least some points, the diseases classically associated with those antibodies were not present, marking such antibodies as clinically insignificant mimickers (likely consequent to inflammatory effects of MCAS on the immune system itself driving spurious/random antibody production) rather than "on-target" and pathogenic antibodies reflecting true disease warranting treatment. We also observed two distinct patterns in trendlines of the titers of the mimickers vs. the trendline pattern expected in a true case of an antibody-associated disease (AAD).
Conclusions: Our observations suggest most positive antibody tests in MCAS patients represent detection of clinically insignificant mimicking antibodies. As such, to reduce incorrect diagnoses of AADs and inappropriate treatment in MCAS patients, caution is warranted in interpreting positive antibody tests in these patients. Except in clinically urgent/emergent situations, patience in determining the trendline of a positive antibody in an MCAS patient, and more carefully assessing whether the AAD is truly present, is to be preferred.

Keywords: antibodies; mast cell activation disease; mast cell activation syndrome; mast cells

Introduction

Though not truly a new disease, the recently recognized disease mast cell activation syndrome (MCAS) arises from chronic inappropriate constitutive and reactive expression of variable subsets of the large repertoire of mediators produced by mast cells (MCs). MCAS thus manifests issues consequent to the effects of these potent mediators. As such, MCAS is a chronic disease (though typically with acute flares upon triggerings of the dysfunctional MCs to further inappropriately activate) with dominant themes of multisystem inflammation ± allergic-type phenomena ± dystrophisms (i.e., aberrancies in growth/development in potentially any tissue) [1]. Due to physician training largely not yet covering MCAS, plus the extreme clinical heterogeneity of MCAS (due to mediator expression heterogeneity from underlying mutational heterogeneity [2–4]), MCAS typically is not diagnosed until years to decades after symptom onset, if ever, despite its reported great prevalence [5, 6]. Both before and after diagnosis of MCAS (as frequently seen with other chronic inflammatory diseases, too), suspicions regarding alternative and/or additional infectious and autoimmune diseases diagnoses arise not uncommonly in patients in whom MCAS is the root issue. Testing for such other diseases often is antibody-based, but the results of such testing (titers, isotypes, and trendlines of these parameters over time) in MCAS patients often are inconsistent with expectations in true cases of such alternative diseases. Such clinically incongruent test results instead more likely reflect inflammatory effects upon the humoral immune system itself, driving spurious production of (1) abnormally high or low (even absent) titers of "legitimate" antibodies [7] (i.e., the antibodies expected to be produced in true instances of the associated infectious or autoimmune diseases) and/or (2) "mimicking" antibodies, whose variable chains sufficiently resemble those of antibodies produced in true instances of

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infectious or autoimmune diseases as to register positive on at least some tests but do not reflect the true presence of the associated diseases. However, many physicians not yet familiar with MCAS, out of an understandable desire to try to help these patients who typically have long sought explanations for their mysterious problems, overinterpret such "positive-yet-atypical" antibody test results as diagnostic of the associated diseases, even when there is scant other supporting evidence. Significant adverse consequences can follow. We have learned to be cautious in interpreting antibody test results in MCAS patients. To illustrate the patterns we have observed, we reviewed the records of some of the MCAS patients from our practices, and we describe the patterns of antibody test results observed in those patients and the lessons learned.

Methods

Case series

Institutional Review Board approval for this IRB-exempt study of anonymized chart records was obtained from Sterling IRB, Atlanta, Georgia (IRB ID 11021). Table S1 in the Online Supplement provides details of 104 cases reviewed by the authors of MCAS patients drawn from their practices who either have been definitively diagnosed per the consensus-2 criteria [1] (73 patients) or are strongly clinically suspected to have MCAS due to their clinical presentation ± some diagnostic testing to date, with further testing pending (31 patients). To help gain a preliminary understanding of the frequency of the "mimicking antibody problem," the first 84 cases in Table S1 constitute the full set of consecutive new patients who consulted with author LBA for suspected MCAS from January 1, 2022 through June 30, 2022. An additional 20 cases (drawn from the practices of authors TTD and GJM) following the first 84 further illustrate how common mimicking antibodies are in MCAS.

Results

The demographics of the MCAS patients reported here are similar to other series (e.g. Ref. [8]). For reasons likely related to both biological and social factors, large majorities of our present series are white (n=95 (91 %)) and female (n=88 (85 %)). Most patients (n=93 (89 %)) first demonstrated MCAS-consistent symptoms no later than adolescence; a large minority (n=41 (39 %)) first demonstrated symptoms in infancy. Also similar to previously published datasets, there was a long delay between onset of symptoms and diagnosis: among the 73 definitively diagnosed patients, and estimating conservatively for when symptoms first emerged in patients who could only provide estimates on that point, the mean, median, and mode intervals between symptom onset and diagnosis were 33, 34, and 45 years, respectively.

One or more antibodies likely to be mimickers (i.e., with clinically incongruent titers and/or isotypes or trendlines of such) were found in a large majority (n=85 (82 %)) of our 104 patients (including 67 (83 %) of the 84 consecutively assessed patients). Most of these 85 patients harboring at least one likely mimicker actually harbored multiple likely mimickers (n=69 (81 %)).

The majority of the infectious disease-targeting antibodies likely to be mimickers which were found (by the authors and/or prior evaluators of these patients) in the patients in our series related to infectious agents frequently causing chronic infection, making such infections reasonable diagnostic considerations for some, but usually far from all, of the chronic symptoms in some MCAS patients. Examples of such infections include viruses (e.g., Epstein Barr virus (EBV), cytomegalovirus (CMV), herpes simplex virus (HSV), varicella zoster virus (VZV), human herpes virus 6 (HHV-6), parvovirus B-19, Coxsackie), tick-borne bacteria and parasites (e.g., *Borrelia*, *Bartonella*, *Babesia*, *Anaplasma*, *Ehrlichia*, *Rickettsia*, *Coxiella*), and certain other bacteria or protozoa (e.g., *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, *Francisella tularensis* and *Toxoplasma gondii*). In the patients in our series in whom polymerase chain reaction (PCR) testing was done to try to confirm the infections suggested by "positive" antibody testing, all such testing was negative. Fluorescent *in situ* hybrid (FISH) testing for classically labeled "tick-borne" bacteria and parasites was positive in a few patients (n=9 (9 %)) with risk factors for such infections (e.g., witnessed tick bites, dog or cat scratches, etc.), though in this small subset of patients there also was much variance in the results from different clinical laboratories using different, often internally developed assays/techniques.

The autoimmune disease-associated antibodies likely to be mimickers which were found most commonly in the patients in our series were anti-nuclear antibodies (ANA), rheumatoid factor, assorted anti-thyroid antibodies, assorted anti-phospholipid antibodies, and anti-IgE or anti-IgE-receptor antibodies.

For example, regarding ANA, 43 of our 104 patients in this series had ANA assessed at least once. Twenty-two (22) of the 43 (51 %) showed no ANA elevations and had negative ANA determined once or more, while 21 (49 %) had ANA positive at least once. Only one of the 21 patients found to have a positive ANA had a high titer (> 1:2,560), but rheumatologic assessment of that patient was that no rheumatologic disease was present (suggesting this antibody was a "pattern B"-type mimicker, as discussed later). The maximum ANA titer in the other 20 patients with at least one

positive ANA was 1:320, and the most common maximum ANA titer was 1:80; 9 of the 21 patients with at least one positive ANA had at least one normal ANA (i.e., in most of the patients in whom anti-nuclear antibodies likely to be mimickers were found, these antibodies seemed to better fit with "pattern A," as discussed later). None of the 21 patients with at least one elevated ANA titer were diagnosed with any rheumatologic disorders. Two other patients were clinically diagnosed with Sjogren's disease despite no supporting laboratory evidence found on testing. In six other patients, a diagnosis of Sjogren's disease was suspected but then refuted upon finding no supporting laboratory evidence. One other patient was found to have positive Sjogren's disease autoantibodies but then assessed by a rheumatologist to not have Sjogren's disease. Two other patients were diagnosed with mixed connective tissue disease based on unknown criteria but did not have elevated anti-U1RNP antibodies. One patient with persistently normal ANA titers was diagnosed with undifferentiated connective tissue disease based on unknown criteria. In 17 of our 104 patients, rheumatoid factor (RF) was assessed once or more; in only two of those 17 (12 %) was RF found elevated once or more in one or more isotypes, always at very modest levels; neither patient was felt by rheumatologic assessment to have rheumatoid arthritis. One patient was diagnosed with lupus based on unknown criteria, and one patient was diagnosed with relapsing polychondritis based on unknown criteria; these diagnoses were then refuted by other rheumatologists. Altogether, 30 of our 104 patients (29 %) underwent one or more rheumatologic consultations, but no rheumatologic diagnosis was clearly established in any of them, and on our own review based on the available records, none of these 104 patients qualified by established criteria for diagnoses of the autoimmune diseases associated with any of the "positive" autoantibodies found in some of them at some points in time. Joint hypermobility history was captured in the 84 patients seen by author LBA; 44 of the 84 (52 %) reported issues with joint hypermobility to one extent or another, but in only two of these 44 (5 %) was a hypermobility spectrum disorder diagnosed, while hypermobile Ehlers Danlos Syndrome, which is often seen in association with MCAS [9] and which is suspected to often be rooted in MCAS [10], was diagnosed in 21 of the 104 patients (20 %, or 48 % of the 44 who reported hypermobility).

Excesses or deficiencies of total levels of the various immunoglobulin classes (IgG, IgM, IgA, IgE, and sometimes even IgD) or subclasses (for IgG and IgA) were found not uncommonly (29 such examples across 19 patients, with mild increases in total IgE or IgA levels unsurprisingly being the first and second most common such examples (7 and 4 instances, respectively), followed by even smaller numbers of instances of other quantitative immunoglobulin class or subclass abnormalities (roughly evenly split between abnormally high levels vs. abnormally low levels)). Such abnormalities were almost always mild in degree and thus likely insignificant. Anecdotally noted is that, similar to observations across the many other MCAS patients in the authors' panels, severe antibody class or subclass abnormality was rarely found in this patient series, just one patient with an undetectable IgA2 level.

Discussion

Immunologists have recognized for decades (e.g. [11, 12]) that although the specific driving mechanisms of such behaviors often remain unclear, the humoral immune system not uncommonly generates antibodies with less than perfect specificity for the intended target. The imperfect specificities of such antibodies are then said to either "cross-react" with "off-target" antigens or "mimic" the "real, on-target" (i.e., more specific) antibodies produced in true instances of the associated infectious or autoimmune diseases. Sometimes such mimickers can be helpful, such as when antibodies imperfectly targeting one strain of influenza virus help protect against other strains. However, imperfect antibody specificity can be troublesome, occasionally driving clinically significant autoimmune disease (e.g., when autoimmune thrombocytopenic purpura arises from imperfectly specific antibodies against urease B in *Helicobacter pylori* cross-reacting with platelet glycoprotein IIIa [13]) and commonly causing diagnostic confusion from falsely positive or falsely negative antibody tests leading to errant diagnosis and treatment of autoimmune or infectious diseases not truly present. Mimicking antibodies are a more common phenomenon than many clinicians may realize; one study in a cohort of reputedly healthy patients found 54.3 % had an elevated ANA (≥ 1:40) [14], and another review suggested ANA positivity rates may be virtually identical between healthy controls and patients [15]. The risks of misdiagnosis arising out of mimicking antibodies would seem to be even greater in chronically mysteriously ill patients (such as most MCAS patients have long been prior to being shown to have MCAS) for whom diagnosticians may be swayed to use misleading "positive" antibody test results to establish "the right answer" for such challenging patients. We suspect our findings likely underestimate both the frequency and range of mimicking antibody issues in MCAS.

MCAS has potential to impact every system in the body (including the humoral immune system) due to the presence of MCs in all tissues and the vast repertoire of potent mediators expressed by MCs. Hundreds of MC mediators have

been catalogued thus far [16, 17], their expression regulated by many factors including genetics [2–4], ligand-binding with the hundreds of mast cell surface receptors catalogued thus far [16], and even various physical forces [18–20]. Most MC mediators have expansive arrays of direct effects and even more expansive arrays of indirect effects. As illustrated by the cases reported here, which echo our experiences with several thousands of other MCAS patients we have seen, several patterns regarding antibody production in MCAS emerge:

(1) Abnormalities in levels of the various classes and subclasses of immunoglobulins, both below and above the normal ranges, are commonly seen in MCAS. However, these abnormalities usually are only mild-moderate in numerical degree and virtually always are clinically insignificant. Furthermore, increased levels virtually always are polyclonal. (Of course, polyclonality cannot be assumed, so when a persistent, moderate or strong elevation in quantitative immunoglobulins of any class or subclass is seen, testing for monoclonality is essential, regardless of whether any monoclonality found is related to the patient's MCAS). Although it has long been known that MC disease is associated with increased risk of hematologic malignancies of all types, few cases of mastocytosis in association with multiple myeloma or even monoclonal gammopathy of undetermined significance (MGUS) have been reported. A search of the PubMed database (https://pubmed.ncbi.nlm.nih.gov) for "mastocytosis myeloma" presently finds only 86 results, and only 11 of those report actual cases of myeloma together with some form of mastocytosis. In our experience, monoclonal gammopathies seem relatively uncommon in patients diagnosed with MCAS, but no studies of the matter have been performed. It seems unlikely that MCAS would be less common in such patients than in the general population. If anything, there is at least a possibility that MCAS might be *more* common in such patients than in the general population. Furthermore, in patients who have not only MCAS but also other co-morbidities, effective treatment of the MCAS concurrently with treatment of a co-morbidity not uncommonly yields better outcome than average/expected for the co-morbidity, raising a question as to whether wider screening for MCAS in patients with monoclonal gammopathies might reveal another pathway for helping to control such diseases.

(2) Perhaps as a matter of inflammation visited by the disease upon the humoral immune system itself, MCAS seems to commonly drive this system into multiple aberrant patterns of production of specific antibodies. Such patterns include the following:

(A) Overproduction of on-target antibodies which are "legitimately" being produced either as drivers of autoimmune disease or in response to true infection (or vaccination).

(B) Underproduction (sometimes even absent production) of on-target, "legitimate" antibodies (such as the low pneumococcal titers found in three of our patients; the true rate of such a finding is unknown since such testing was not systematically done in these patients) which should be produced in response to true infection (or vaccination).

(C) Likely most prolifically by far, antibodies which are "illegitimate" to produce in that they are not being produced in response to any apparent provocative/stimulatory antigen. The Fab regions of most such "illegitimate" antibodies likely have such poor specificity that they will never be detected, but some of these "illegitimate" antibodies – likely only a small fraction – sufficiently resemble/mimic "on-target" antibodies that they register "positive" on at least some of the tests for detecting such antibodies. However, "mimicking" antibodies manifest immunoglobulin isotype and titer patterns often distinct from the isotype and titer patterns classically seen with legitimate on-target antibodies. Our experience suggests the results of repeated tests over time for any given mimicking antibody produced in an MCAS patient follow either of two patterns, as illustrated in Figure 1, and specific examples of these patterns, drawn from the 104-patient dataset detailed in Online Supplement Table S1, are shown in Table 1. Regardless of which of these two titer trendlines any given mimicking antibody demonstrates, mimickers show few to none of the classic clinical manifestations of the disease associated with the on-target antibody the mimicking antibody resembles. For example, it is common in MCAS to see modest waxing/waning levels of antithyroid antibodies and yet see little to no aberrancies in thyroid function tests and no correlation of the thyroid function test abnormalities with the extent of the fatigue often reported by the MCAS patient. Less commonly, persistently off-the-scale titers of an antithyroid antibody may be seen without any corresponding aberrancies in thyroid function testing or imaging. As another example, persistently very high titers of antibodies seemingly targeting various Epstein Barr virus (EBV) antigens may be found, albeit in target-antigen and immunoglobulin isotype class patterns not clearly consistent with either new or past EBV infection, and with negative EBV viral load studies by PCR ruling out current EBV infection.

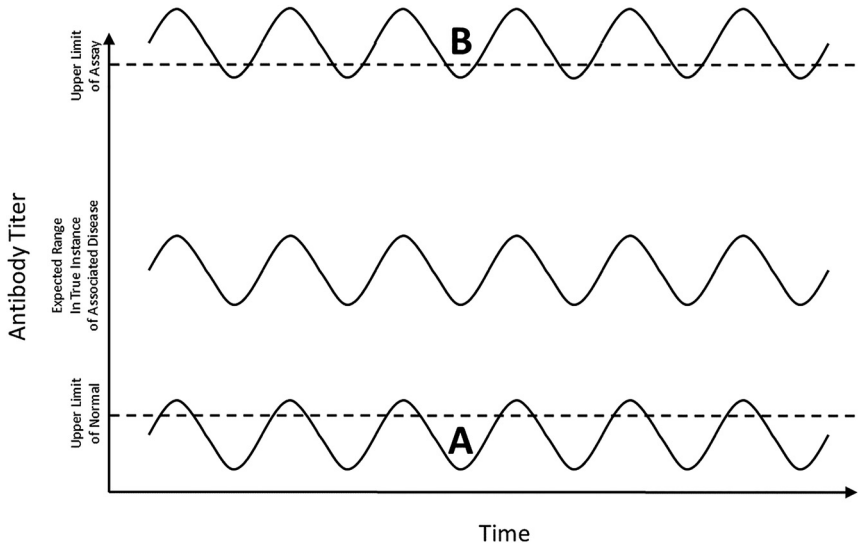

Figure 1: Common patterns of mimicking antibody test results in MCAS patients. The unlabeled antibody titer pattern over time in the middle indicates the typical variance of the titer, relative to the upper limit of normal and the upper limit of the assay, in a true (i.e., clinically obvious) instance of the disease associated with the antibody being measured. Pattern A shows the more common pattern observed over time with mimicking antibodies in MCAS patients, with "positive" titers never rising more than modestly above the upper limit of normal, and with the titer frequently in the normal/"negative" range. Pattern B shows the less common pattern observed over time with mimicking antibodies in MCAS patients, with persistently extremely elevated levels (often "off-the-scale"). In addition to the abnormal antibody titer patterns (either A or B) seen over time with mimicking antibodies in MCAS patients, and as another hint that the observed titers are measures of a (clinically insignificant) mimicking antibody reflecting effects of inflammation on the humoral immune system, the typical clinical presentation of the disease associated with the antibody (and at the severity expected in accordance with the observed titers) is not seen.

MCAS patients usually have been multisystemically ill in "mysterious" fashion for years to decades prior to being diagnosed with MCAS. The temptation in the physician attending to a chronically mysteriously ill patient to "save" the patient and finally establish an accurate diagnosis leading to significantly helpful treatment can be strong. In our experience, this temptation not uncommonly – and not unreasonably – leads to much testing by many diagnosticians over time for large assortments of specific antibodies. However, when underlying MCAS and the mimicking antibody issues described herein are not known/recognized by the physician and the patient is then diagnosed by the well-intentioned physician with the disease which is associated with the "positive" antibody test (even though the physician knows the clinical pattern of illness expected based on the test result is not present), the consequences of (mis)treatments for such (mis)diagnoses can be inconvenient at best and dire at worst in medical, financial, and other manners.

Although clinicians unquestionably should follow "clinical instinct" and issue preliminary diagnoses and treatments in dire circumstances (e.g., clinically apparent sepsis, catastrophic anti-phospholipid antibody syndrome, etc.), we have learned it is best to be cautious in interpreting most "positive" results on specific antibody tests in MCAS patients and be hesitant to diagnose, much less initiate treatment for, the disease associated with a "positive" such test unless three conditions are met, as detailed in Table 2.

Short of meeting all three of these criteria, the physician attending to an MCAS patient may better serve the patient by being cautious about an observed "positive" antibody test result and delaying diagnosis and treatment of the antibody-associated disease. Caution may well be the better part of valor in taking time (possibly several months to even a year or two) to follow repeat testings (roughly every 3–6 months) to clarify whether the trendlines of the antibody and the patient's clinical behavior are more consistent with the trendlines expected of a mimicker vs. an on-target antibody.

3. We additionally mention our similar observations (i.e., frequent findings of mildly high or low levels; specific data not shown) regarding many other metabolic parameters in MCAS, such as various aspects of thyroid or adrenal function, or iron-related parameters or tumor markers, allegedly signaling presence of thyroid disease or adrenal or iron deficiency or tumor despite no clear causes or consequences of thyroid or adrenal disease or iron deficiency, and no other clear evidence of tumor, ever being found. Similarly, sufficient evaluation of MCAS patients frequently

Table 1: Examples of mimicking antibody patterns in MCAS patients. (Case numbers refer to cases listed in online supplement Table S1).

(1) Specific antibodies waxing/waning between normal titers and mildly elevated titers, and no past or present clinical evidence of the associated disease:
   a. Anti-IgE-receptor antibodies: Cases 5, 11, 32
   b. Anti-nuclear antibodies (ANA): Cases 8, 12, 30, 38, 52, 66, 72
   c. Celiac disease antibodies: Case 28
   d. Anti-neutrophil-cytoplasmic (ANCA) antibodies: Case 40
   e. Anti-IgE antibodies: Case 54
   f. *Mycoplasma pneumoniae* IgG: Case 60
   g. *Borrelia burgdorferi* IgG: Case 64
   h. Anti-thyroid-peroxidase (TPO) antibodies: Case 81
   i. Anti-thyroglobulin antibodies: Case 81
   j. Rapid plasma reagent (anti-*Treponema pallidum* antibodies): Case 50
   k. *Ehrlichia chaffenssi* IgG: Case 86
   l. *Anaplasma phagocytophilia* IgG: Case 86
   m. Lyme IgM Western blot band 41: Case 86
(2) Specific antibodies mildly elevated, but no past or present clinical evidence of the associated disease:
   a. Anti-IgE-receptor antibodies: Case 1
   b. Anti-TPO antibodies: Cases 23, 37, 50, 87
   c. Rheumatoid factor: Cases 28, 36, 104
   d. Anti-adrenal antibodies: Case 40
   e. IgG antibodies to assorted Epstein Barr virus (EBV) antigens: Cases 4, 6, 9, 14, 18, 24, 36, 37, 48, 67, 83, 85, 89, 92, 93, 95, 99, 100, 101, 103
   f. IgM antibodies to assorted EBV antigens: Case 85
   g. IgG antibodies to assorted *Toxoplasma gondii* antigens: Cases 11, 14, 18, 19, 21, 22, 24, 26, 27, 31, 37, 38, 41, 42, 44, 45, 46, 49, 51, 52, 53, 54, 55, 56, 57, 63
(3) Specific antibodies extremely elevated, but no past or present clinical evidence of the associated disease:
   a. Anti-IgE-receptor antibodies: Case 65
   b. ANA antibodies: Case 85
   c. HSV-1/2 IgG antibodies: Case 103
   d. EBV IgG antibodies: Case 93
   e. Anti-thyroglobulin antibodies: Case 32

Table 2: Recommended criteria for assessing a "positive" antibody titer in an MCAS Patient as indicative of a true instance of the associated disease.

(1) The specific antibody expected to be present in the infectious or autoimmune disease with which the antibody is associated should be persistently present at a relatively stable titer at a level consistent with the observed severity of the suspected associated disease. Furthermore, the presence of the antibody preferably should be confirmed at more than one clinical laboratory of generally good reputation for high-quality testing in the applicable domain. The statistical anomalies represented by "outlier" positive results should be considered as possibly reflecting the use of antibody-based probes which themselves are of insufficient specificity and thus may more often detect mimicking antibodies. Additional supporting evidence should be sought to support a diagnosis suggested by a single outlier positive result on any antibody test in an MCAS patient.
(2) The clinical disease classically associated with the "positive" antibody should be clearly present, and in a fashion consistent with the observed antibody classes and titers seen over time.
(3) Any non-antibody-based tests (e.g., cultures or PCR or FISH tests for detecting infection) which are available for confirming the presence of the disease associated with the "positive" antibody test should also be persistently positive in manners aligned with the observed clinical behavior of the associated disease, and preferably confirmed at more than one clinical laboratory of generally good reputation for high-quality performance of such tests.

complaining of odd neurologic, dermatologic, or abdominal pain presentations not uncommonly finds them to have modest elevations in various porphyrin fractions, sometimes leading to diagnosis and even treatment for various forms of porphyria despite the facts that (a) the totality of the patient's presentation virtually never is particularly consistent with the alleged porphyria diagnosis, and (b) the pattern (in terms of specific porphyrin fractions and levels of those fractions) of porphyrin elevation is not at all consistent with any known type of porphyria. Patients who truly have porphyria usually have baseline levels of the relevant porphyrin fractions (i.e., in specific patterns among the various fractions, in accordance with specific types of porphyric disease) consistently at least 2–3 fold above the upper limits of normal for those tests, spiking to 5–10 fold elevations or even much higher during acute attacks, whereas patients with MCAS not uncommonly have quite modest elevations in various porphyrin fractions (almost always well below two-fold above the upper limits of normal) and in porphyrin fraction patterns not well fitting with any known type of porphyria. Various porphyrin fractions naturally elevate modestly in response to any stress, and it is hard to imagine how a decades-long, multisystem, multisymptom disease such as MCAS (causing frank disability in a non-trivial number of patients) cannot cause significant physical and psychological stress (which unfortunately only "feeds back" and further aggravates/triggers not only MCAS but also intermittent modest escalations in various porphyrin fractions, clearly a reactive phenomenon quite different from true porphyria of any type).

To be clear, development of one disease (e.g., MCAS) does not make it impossible for other disease (e.g., infection, autoimmunity) to also develop. Although MCAS can drive a vast array of symptoms, and though it is tempting to MCAS patients and their providers alike to reflexively attribute every new problem in such patients to their MCAS, patient and physician alike nevertheless are better served by being

consistently self-disciplined and diligent in carefully evaluating any new symptoms which emerge (or any old symptoms which worsen in unusual fashions) to rule in or out as accurately as possible other diseases which seem to well fit the new/worsened symptoms. For example, in some parts of the world Lyme disease is practically epidemic, and it has long been recognized that the causative *Borrelia burgdorferi* bacteria can trigger activation of MCs, as would be expected with virtually any type of infection. Therefore, MCAS patients who acquire true Lyme disease need to be identified as such, though the challenges with interpreting antibody-based testing in MCAS patients would seem to make tests which probe for the DNA sequences unique to such infectants (e.g., PCR or FISH tests) the preferable diagnostic approach in MCAS patients. Such patients then should be effectively treated for the infection, since otherwise the chronic active infection likely will forever continue serving as a significant aggravating factor for the MCAS and forever continue limiting the efficacy of any MCAS-directed therapies. However, if the patient's overall lifetime medical history is more consistent with primary MCAS which was already somewhat symptomatic at baseline and then came to be further aggravated/escalated by acquisition of Lyme disease (rather than the patient having been consistently healthy until acquisition of *Borrelia* infection, with subsequent onset of symptoms consistent with (purely secondary) MC activation), then a failure of "Lyme disease symptoms" to resolve despite proven eradication of active *Borrelia* infection could be recognized as a persistence of the (incurable) primary MCAS rather than as failure of Lyme disease treatment and persistence of "chronic Lyme disease." (Also, other vector-borne infections sometimes accompany *Borrelia* transmission (e.g., *Bartonella*, *Babesia*), so a search for such after eradication of *Borrelia* would be reasonable while MCAS treatment continues to be pursued.) In this vein, and with the above-noted exceptions for imminently life-threatening situations in mind, antibody-based testing for various infectious or autoimmune diseases will be appropriate from time to time in MCAS patients, but when the "positive" results of such testing nevertheless do not fit well with the observed clinical situation, caution (on the parts of both the physician and the patient) usually is advisable.

Though a number of insights have been gained thus far as to likely relevant anatomic relationships (e.g. [21–26]) and mediator-based mechanisms (e.g. [27–34]) by which MCs might directly and/or indirectly contribute to the development of the arrays of humoral immune system aberrancies seen in MCAS, overall this is a largely uninvestigated area. However, the known expression by MCs of a great many mediators (chemokines, interleukins, etc.) for which receptors are known to be present on various lymphocyte populations [35, 36], including T cells, B cells, and plasma cells, plus the proximity of MCs to lymphocytes and plasma cells in many tissues, strongly suggest many such mechanisms exist – and the readily apparent extreme clinical heterogeneity of MCAS suggests correspondingly great heterogeneity in the specific assortments of humoral immune system aberrancies MCAS may drive in different patients.

Conclusions

Although quantitative immunoglobulin testing and specific antibody testing often are warranted in MCAS patients due to their complex multisystem clinical presentations with a wide variety of chronic inflammatory and other issues which may signal the presence of comorbidities whose clinical profiles partially overlap the profiles MCAS itself can produce, the humoral immune system in MCAS often spuriously produces legitimate antibodies in aberrant patterns and also often spuriously produces mimicking and cross-reactive antibodies. Although no tests are yet available in clinical laboratories for distinguishing on-target antibodies of clinical significance from mimicking/cross-reactive antibodies of no clinical significance, the clinical approaches described above often do permit such distinctions to be made and thereby may be able to help physicians and patients avoid important misdiagnoses and mistreatments.

Research ethics: Not applicable.
Informed consent: Not applicable.
Author contributions: LBA conceived this work, wrote the manuscript, and contributed cases. GJM and TTD contributed cases. GJM participated in the editing of the manuscript.
Competing interests: LBA is an uncompensated member of the medical advisory board for MC Sciences, a pharmaceutical firm developing novel therapies for mast cell disorders. TTD declares no conflicts of interest. GJM is the co-founder and chief medical officer of MC Sciences. All author have accepted responsibility for the entire content of this manuscript and approved its submission.
Research funding: No funding was received for performance of the research reported here or for the writing of this report, but the open publication of this work was supported by a grant from the Förderclub Mastzellforschung e.V. (Germany).
Data availability: All of the data for this study are provided in Online Supplement Table S1.

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Supplementary Material: This article contains supplementary material (https://doi.org/10.1515/dx-2023-0032).