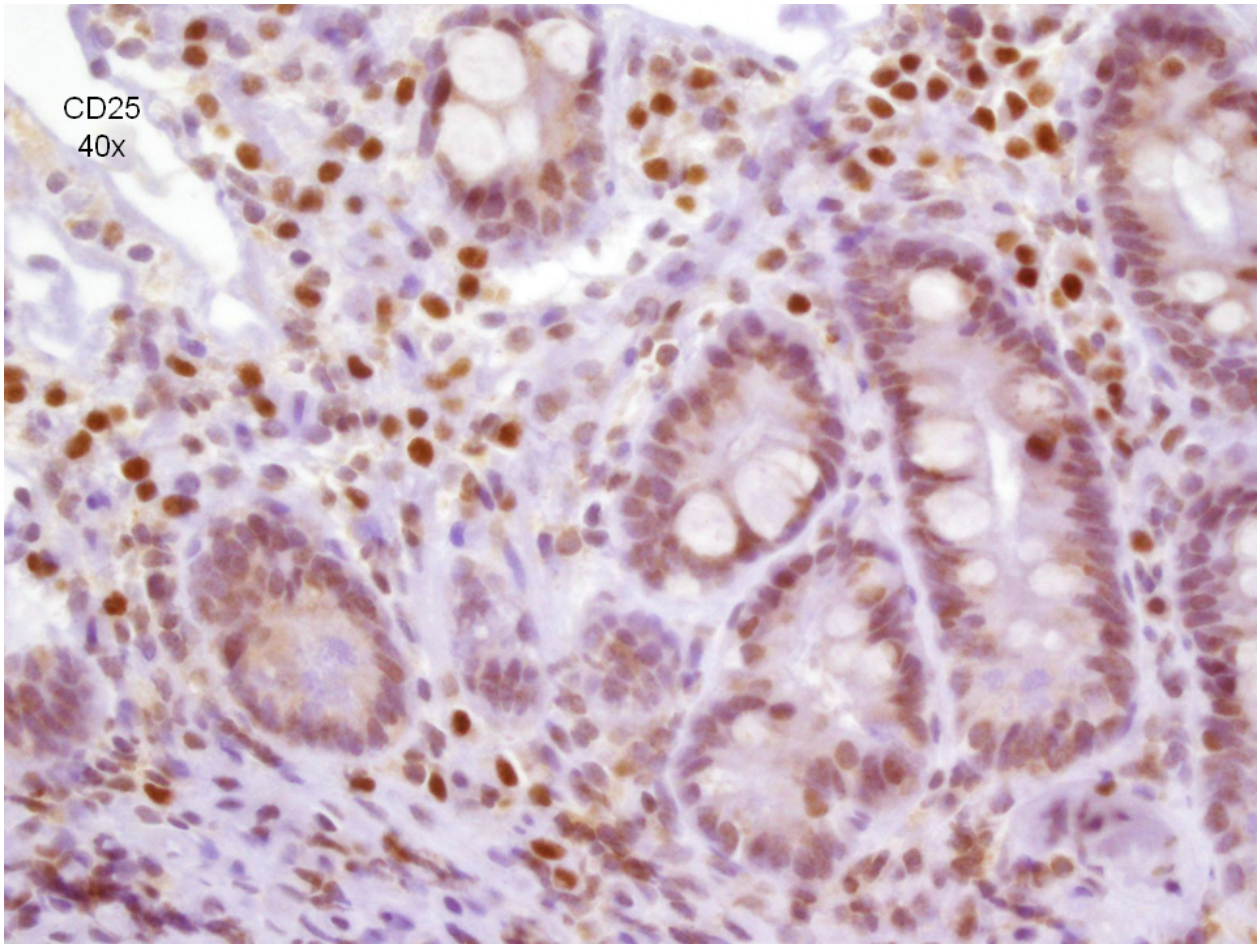


The pathologist called me 3 weeks later and was at a loss for words. “I’ve never seen anything like this before. The H&E stain was completely textbook normal, but the immunohistochemistry stain was completely abnormal.”

These were the biopsies. [Slides 13 and 14] This was the routine staining with H&E, and these results are normal. This is what normal duodenal mucosa is supposed to look like on H&E staining. [Slide 15]. And this was the abnormal staining. Many of the cells that had been thought by the pathologist to be lymphocytes actually were mast cells instead. This is the CD117 stain. [Slide 16] And this is the CD25 stain.

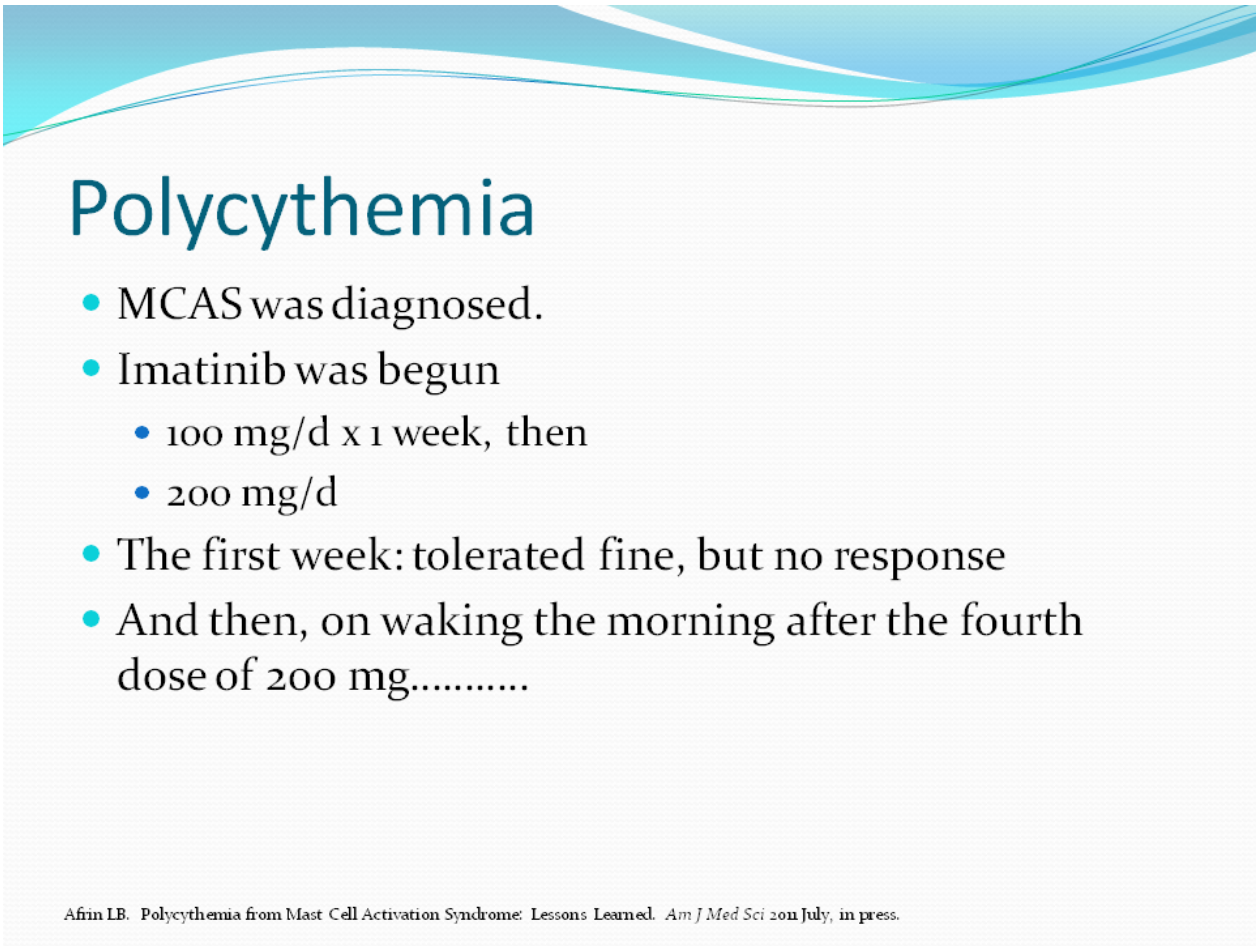
Dr. Trinkaus: Sorry, a very quick question. The H&E stain, if he’d done Giemsa or other mast cell stains, would that have been positive?

Dr. Afrin: Possibly.

Dr. Trinkaus: Possibly?

Dr. Afrin: What I have found in my experience in the last 3 years is that if you've got to pick one stain, the CD117 is the probably the best stain to go with. I prefer to do a battery of stains if possible, but it's not always possible.

[Slide 17: Polycythemia.]



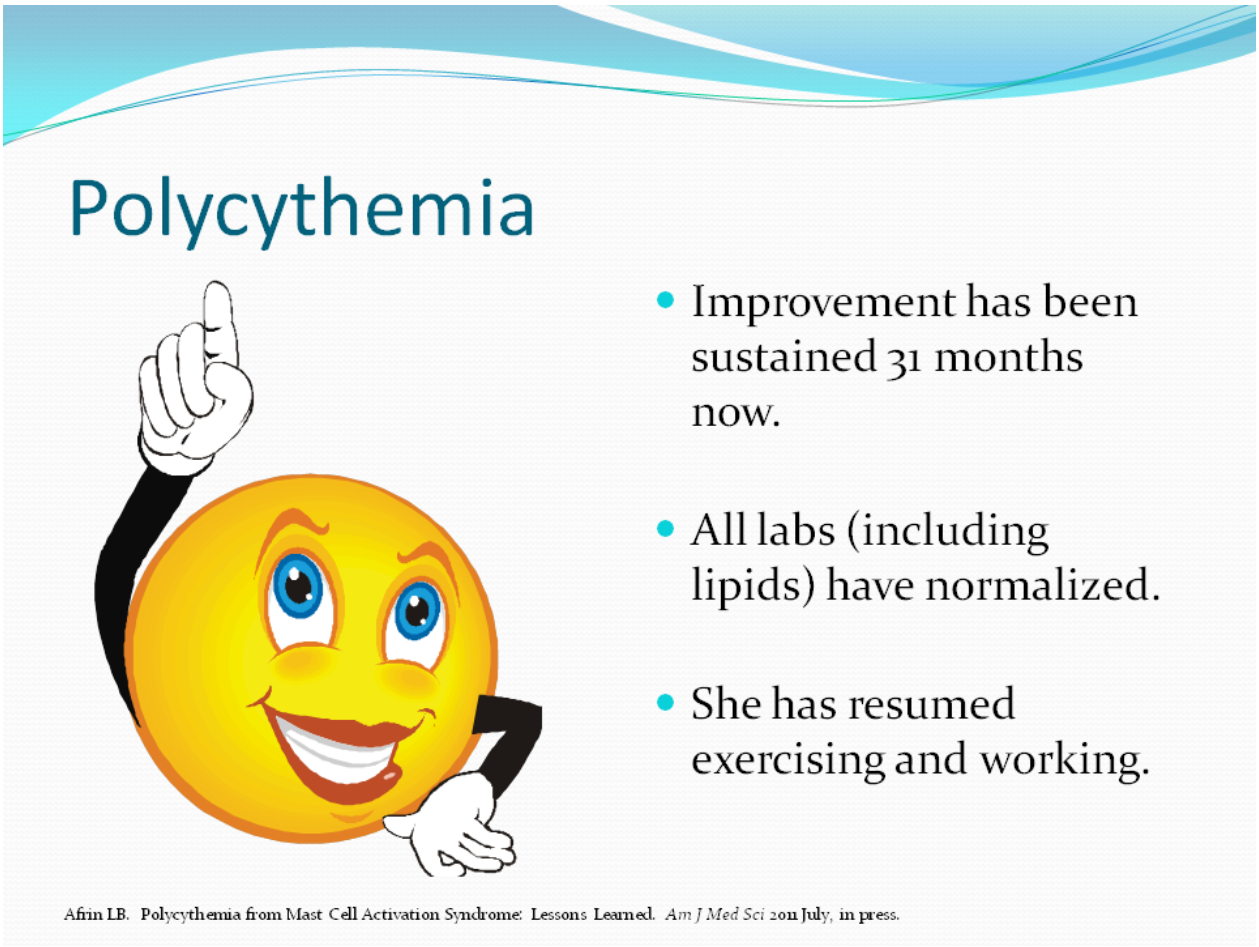
I called the patient back into the office and told her, "I think we have identified the root of your problem as mast cell disease, but the form of mast cell disease that appears to be in you doesn't match any diagnostic criteria we've got. I don't know what to call this diagnostically, and from a treatment perspective, there are no guidelines available, either." We talked about a lot of options, and in my naïveté at the time I decided to recommend a trial of Gleevec (imatinib) as her first-line therapy.

Dr. Sibbald: I want you to tell them what Gleevec is.

Dr. Afrin: Gleevec is a tyrosine kinase inhibitor, but we're going to get into these biologic details shortly, so I'd like to defer that for now.

So I started her at the lowest available dose and instructed her to double it after a week if she were tolerating it OK but hadn't noticed any improvement.

[Slide 18: Polycythemia.]

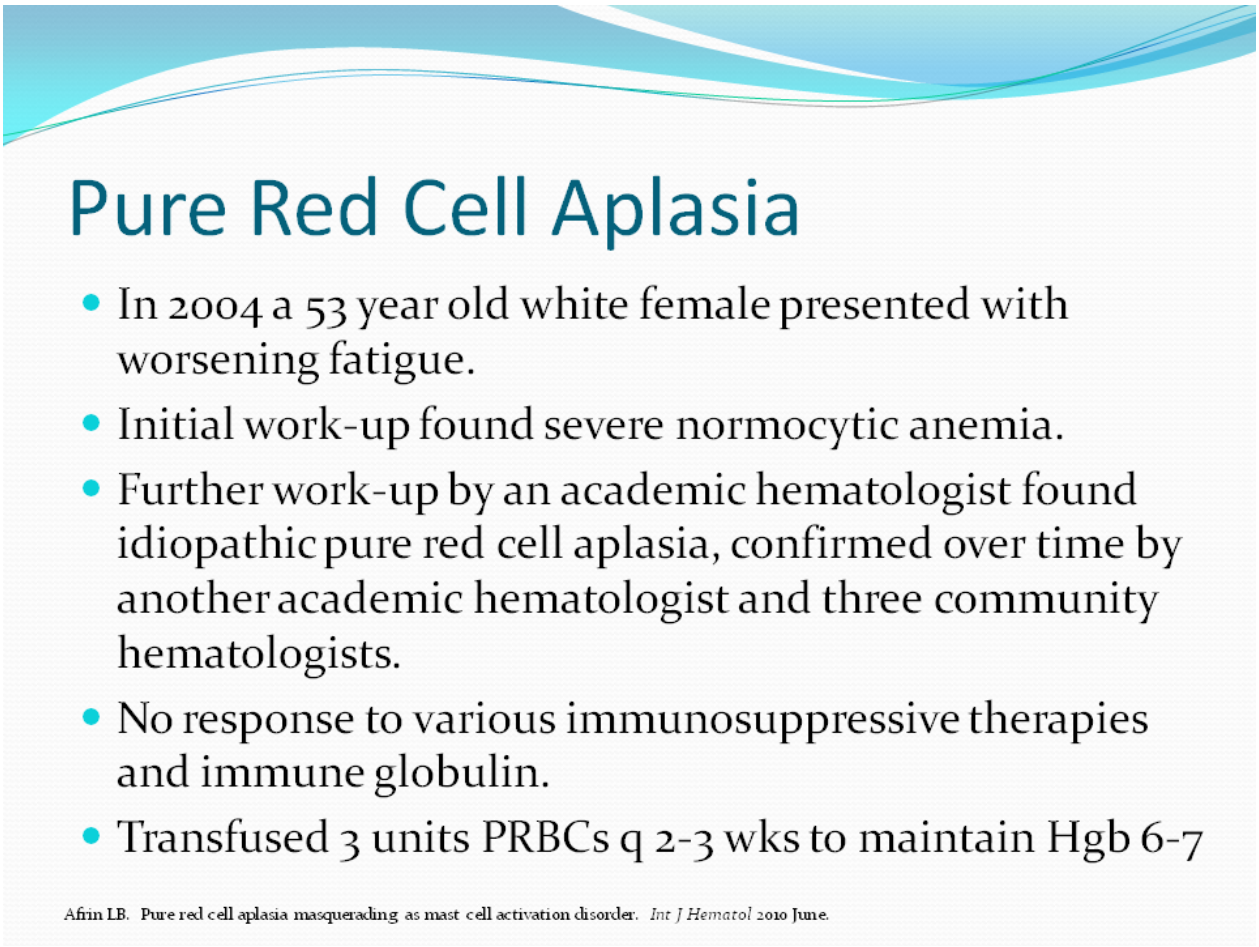


She returned a month later. When I walked in the exam room, the difference was stark. She was beaming, a big smile on her face; she was radiating energy. I asked, "What happened?" She said, "The first week, nothing happened. So then I doubled the dose, as you said." She woke on the morning of the 5th day of the doubled dose, and she was acutely aware upon waking that morning – she hadn't even gotten out of bed, she had just opened her eyes – that all of her symptoms were gone.

She's been running around ever since, just as energetically as she had been before she started getting sick. She has put her business back together, she's back to exercising, she has her old life back. She forgot her dose once about 3 months into this. She got punished for that about 18 hours later when her malaise came back in force. So she quickly took the dose she had missed, and about 12 hours later things settled back down. She has had an occasional incident since then of a mild relapse of malaise. She has learned to just pop an extra Gleevec when that happens and the extra dose reliably, quickly, completely quells the spell. Now, it's important to understand that what I'm describing to you is, as I've learned since this serendipitous first encounter with MCAS,

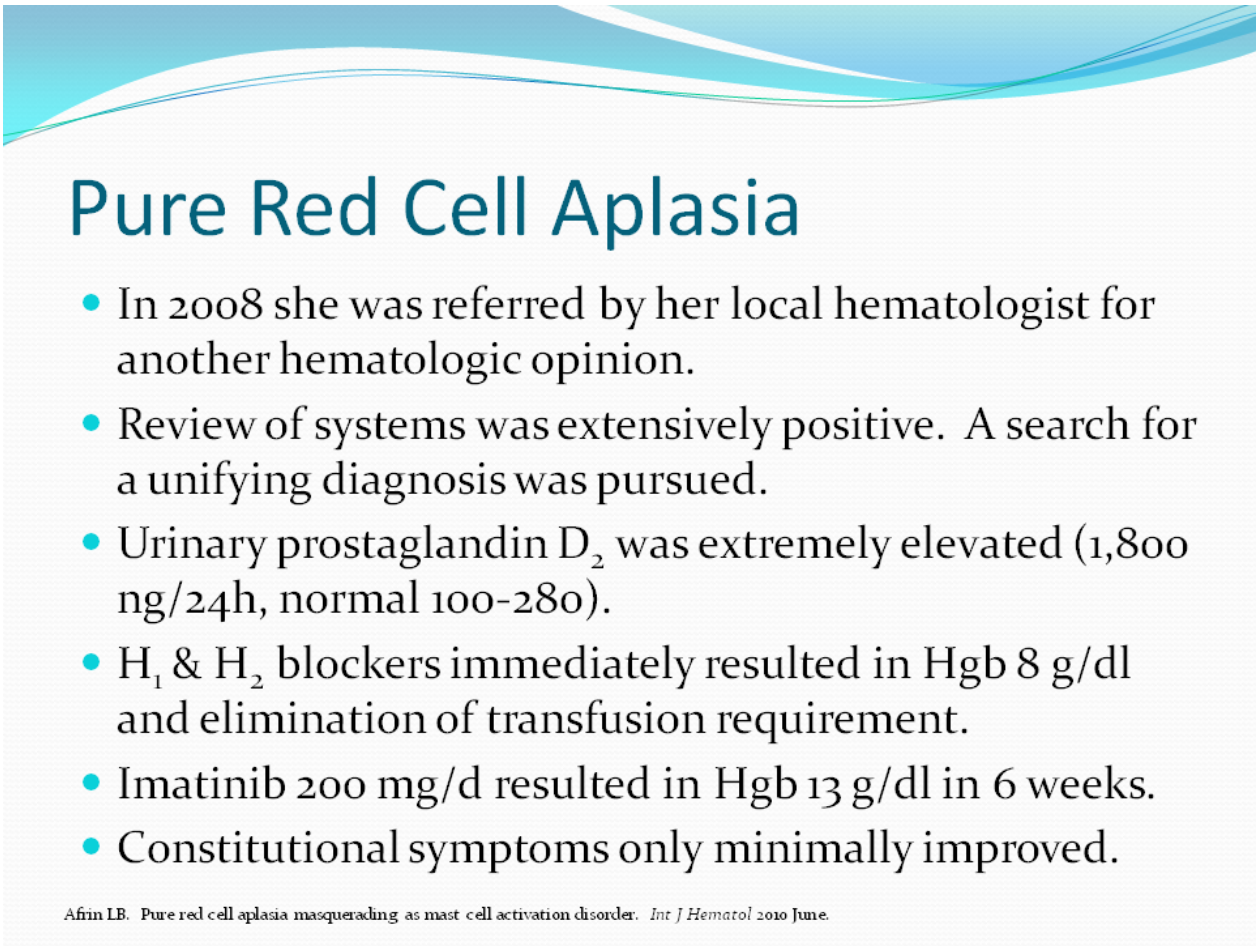
an atypical response to Gleevec. I will show you other nice responses to Gleevec, but this one is atypical with respect to how complete the response was.

[Slide 19: Pure Red Cell Aplasia.]



Now, on to virtually a polar opposite case in terms of presentation. This is the 3rd patient I diagnosed with MCAS. In 2004 a 53 year old white female presented with worsening fatigue. She was found to have severe anemia, and she was certified by multiple academic and community hematologists as having a pretty rare disease called pure red cell aplasia (PRCA), and furthermore this was a form of PRCA which was not due to parvovirus B19. She had had no response to all the standard therapies for PRCA. She became heavily transfusion dependent.

[Slide 20: Pure Red Cell Aplasia.]

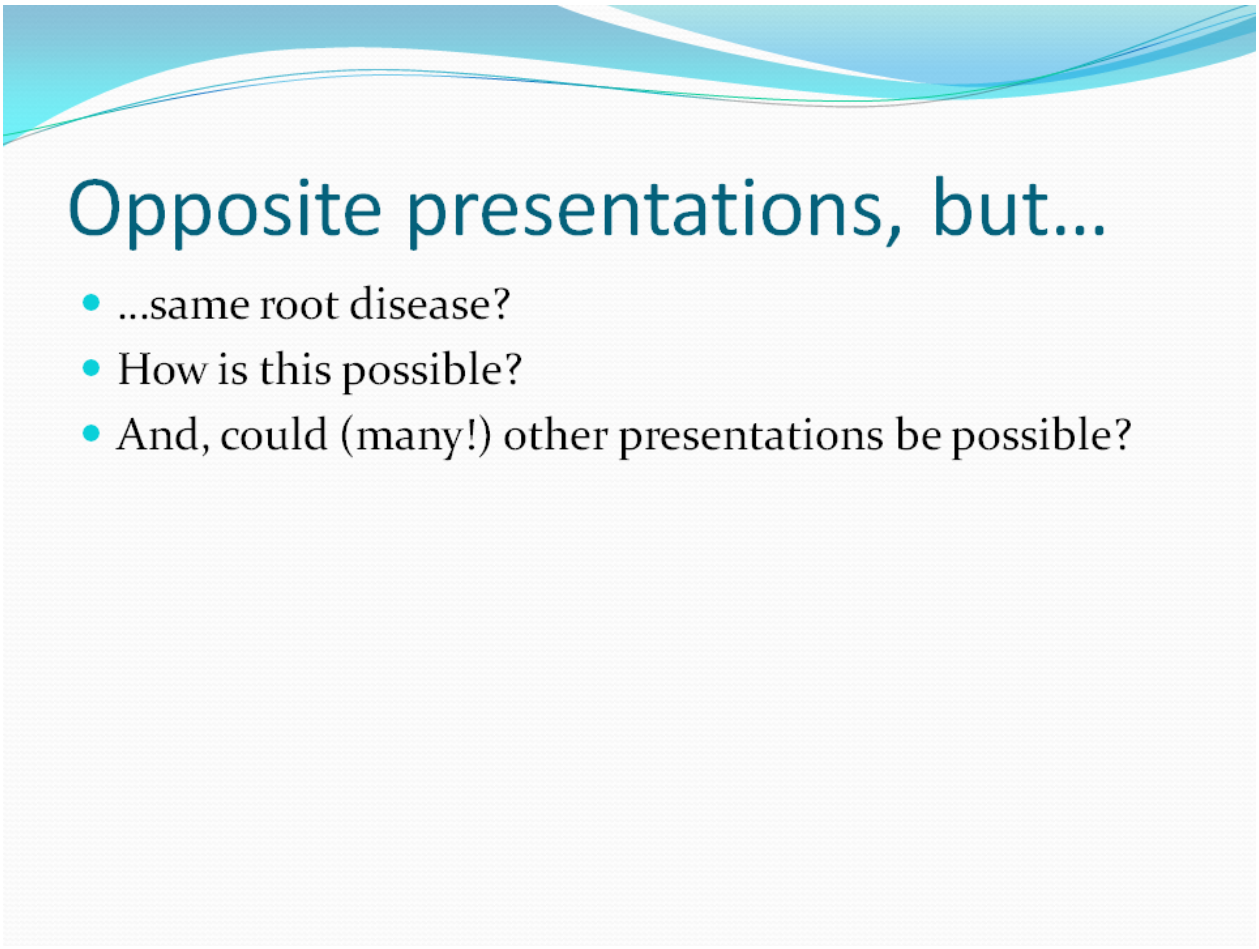


Somehow she got referred to me in 2008 for another opinion. And again, a careful history quickly revealed there was no way this could be PRCA because she had a large number of symptoms which could not be explained by PRCA or any of her other known conditions. So it again became a quest for what she could have that had decimated her red cell production and caused all of her other problems. From what I'd been reading about what mast cells are capable of doing, I began to wonder whether it might be possible for one patient to have one variant of mast cell disease that releases mediators which suppress red cell production while another patient has another variant that releases mediators which increase red cell production.

So I did some testing which revealed her to have a very high PGD_2 level which still today is the highest PGD_2 level I've ever seen. I had learned more about treatment by that point, so I did not immediately start her on Gleevec. I started her on antihistamines and immediately eliminated her transfusion requirement, but her hemoglobin persisted around 8 g/dl. I then added low-dose Gleevec, and she had complete normalization of her hemoglobin within 6 weeks. This is actually the first of my MCAS cases that I

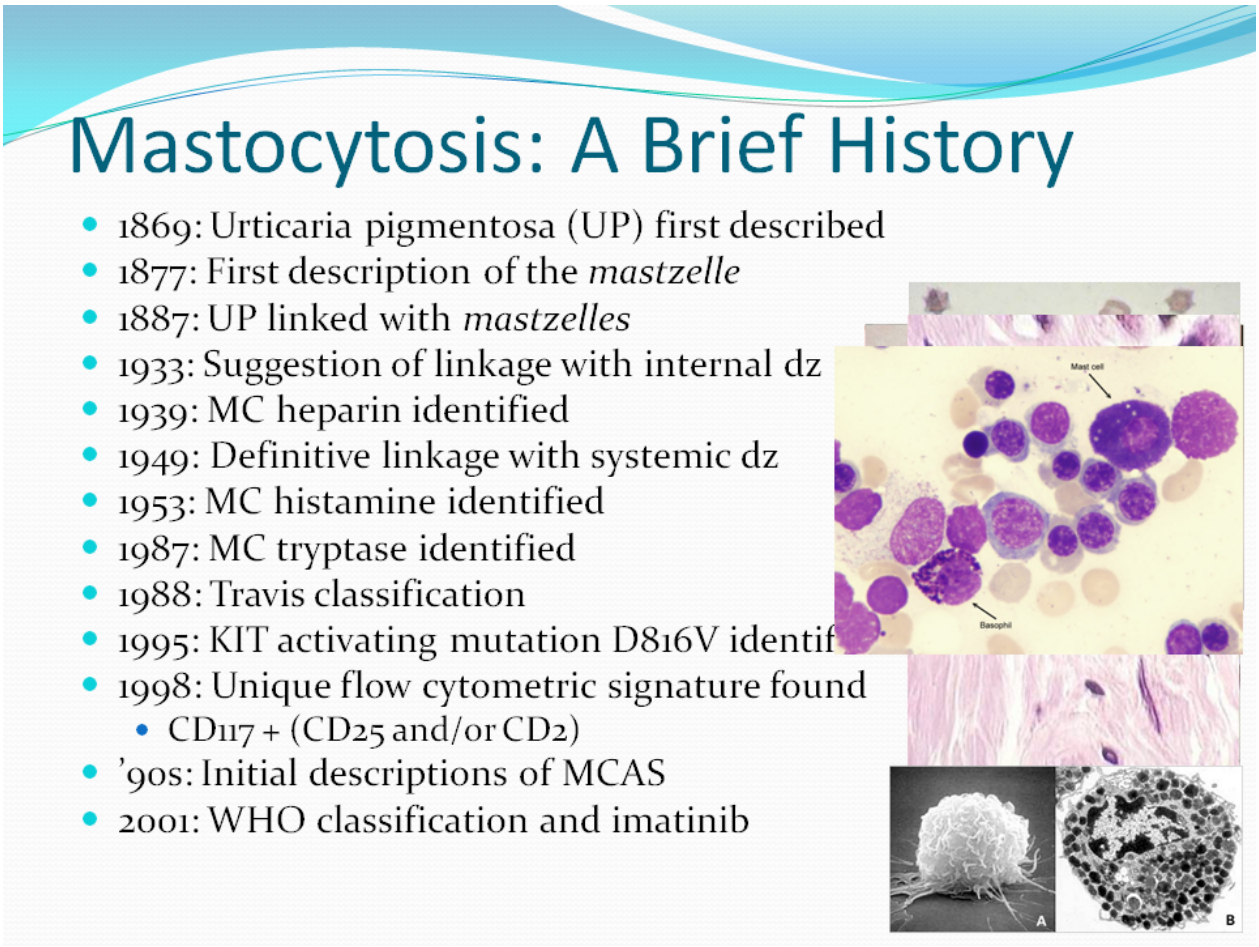
published, appearing in the *International Journal of Hematology* last summer (2010). Unfortunately, her constitutional symptoms have only been minimally improved. It's clear that Gleevec is not controlling the entirety of the disease, and we continue to search for other means of treatment for her symptoms.

[Slide 21: Opposite presentations, but…]



So, opposite presentations (polycythemia and PRCA), but seemingly the same root disease. How is this possible, and is it possible that many other presentations could be out there?

[Slide 22: Mastocytosis: A Brief History.]



Let's digress for a moment to the history of mastocytosis.

This is a disease of mast cells that we've known about for the last 140 years. Urticaria pigmentosa was first described in the late 1860's and was linked with the mast cell – a "well-fed" heavily granulated metachromatic cell – described by Paul Ehrlich in the late 1870's. Fifty years later the cell was linked to internal, systemic disease. Then we started finding the mediators that mast cells release – heparin, histamine, tryptase. The first classification of mast cell disease was produced in the late 1980's, shortly followed by identification of the mutation(KIT-D816V) which has now been found in more than 90% of cases of systemic mastocytosis. Just a few years later, Dr. Escribano and colleagues in Spain identified a unique flow cytometric signature – CD117 co-expressed with CD25 or CD2 – that seems to be pathognomonic for mast cell disease. It was actually in the early 1990's in an article by Jack Roberts and John Oates in the *Journal of Investigative Dermatology* where you see the first suggestion of what has come to be called mast cell activation syndrome (MCAS). I'm obviously paraphrasing here, but what they basically said was, "We know there are patients who behave like they have

mast cell disease, and we can find elevation in some mediators in some of these patients, but in other patients we can't find any elevated mediators. Also, we often have a hard time finding the aberrant mast cells themselves (above and beyond finding the abnormal mediator levels). In summary, we think there is a disease here, but we just can't yet characterize it better".

Then in 2001 the first World Health Organization classification of Systemic Mast Cell Diseases was released. That's also the year when Gleevec became available, and we soon learned that the dominant mutation in systemic mastocytosis – the KIT-D816V mutation – by and large is resistant to Gleevec. To be sure, there indeed are some patients with KIT-D816V who respond to Gleevec, and we don't have reasons for that at the moment. But, by and large, imatinib is not going to work for patients with systemic mastocytosis. (However, I'm going to show you some information in a second that there is a whole lot more to mast cell disease than systemic mastocytosis.)

Dr. Sibbald: Just out of curiosity, around the room, the other physicians, what have you experienced in terms of mast cell disease? In terms of the spectrum that you've seen? I have a tremendous number of these patients, and I would say that by and large the ones that I see are mostly cutaneous, with some systemic symptoms. There seems to be a big overlap with the physical urticarias manifesting dermatographic and cholinergic responses to heat, exercise, and emotion, and lots of histamine-releasing drugs appear to act as triggers. We don't see too many with the systemic disease, but I'm curious about your perspective.

Dr. Trinkaus: A lot of these patients come to us because they have very severe anaphylaxis and constitutional symptoms including overwhelming fatigue, stomach upset, and nausea. So our practice is just exactly what you've been doing except for the urine PGD_2 test. I don't think we have access to that at our hospital, and that's something I plan to look into because in one in seven of the patients we test for mast cell disease, all the tests are negative and this poor patient is just sitting there in diagnostic limbo. Some of them plead for me to "just do something for them," and it's just so overwhelmingly impossible to deal with. And they're on everything. We have had one mast cell leukemia and that person also had the KIT-D816V mutation. We tried Gleevec, but unfortunately he passed away. I probably see one or two mast cell disease patients a month. I only started about 2 years ago, but I see about 3 patients that have legitimate systemic mastocytosis, including one in whom it just spontaneously resolved.

Dr. Sibbald: My patients, too, usually have normal tryptase levels.

Dr. Trinkaus: Yes, lots of ours have normal tryptase.

Dr. Afrin: We'll get to more discussion on that aspect in a moment.

Dr. Monthropey: I'm sort of new as a dermatologist, so my first mastocytosis patient was today. At least, I'm pretty sure it's mastocytosis. I sent her for a serum tryptase, and I'm not sure how that's going to come back. I'm wondering if I should have tried to send her for a urinary PGD_2 because I think I think Sunnybrook Hospital may actually be able to do that test (but I'm not sure).

Dr. Sibbald: It's not very easy to get the PGD_2 urine test. We can't get them. Marsha?? I don't think I've been able to get any of those.

Dr. Trinkaus: Neither can I.

Dr. Sibbald: You know, the prostaglandins are so short-lived and require carefully controlled conditions for harvesting and processing. Marsha, your experience in endocrinology?

Dr. Werb: Well, actually they come usually looking for a pheochromocytoma or carcinoid, and they come in with very variable symptoms. I'm more used to thinking about glucagonomas when I see the kind of migratory erythematous rash often seen in mast cell disease. But my one or two cases that I'm involved with, as soon as they've been discovered to have mast cell disease, they leave me because they don't have an endocrine disorder, so how they get treated is very interesting to me, especially about Gleevec. You said the KIT-D816V mutation (predicting for resistance to Gleevec) is absent in 10% of these patients?

Dr. Afrin: Yes, but that's 10% just in systemic mastocytosis, and I'll be speaking more later about mast cell activation syndrome, which is the far more prevalent problem.

Shelley: I'm also interested in this because I have had, and continue to have, endocrine problems, and I also have mastocytosis.

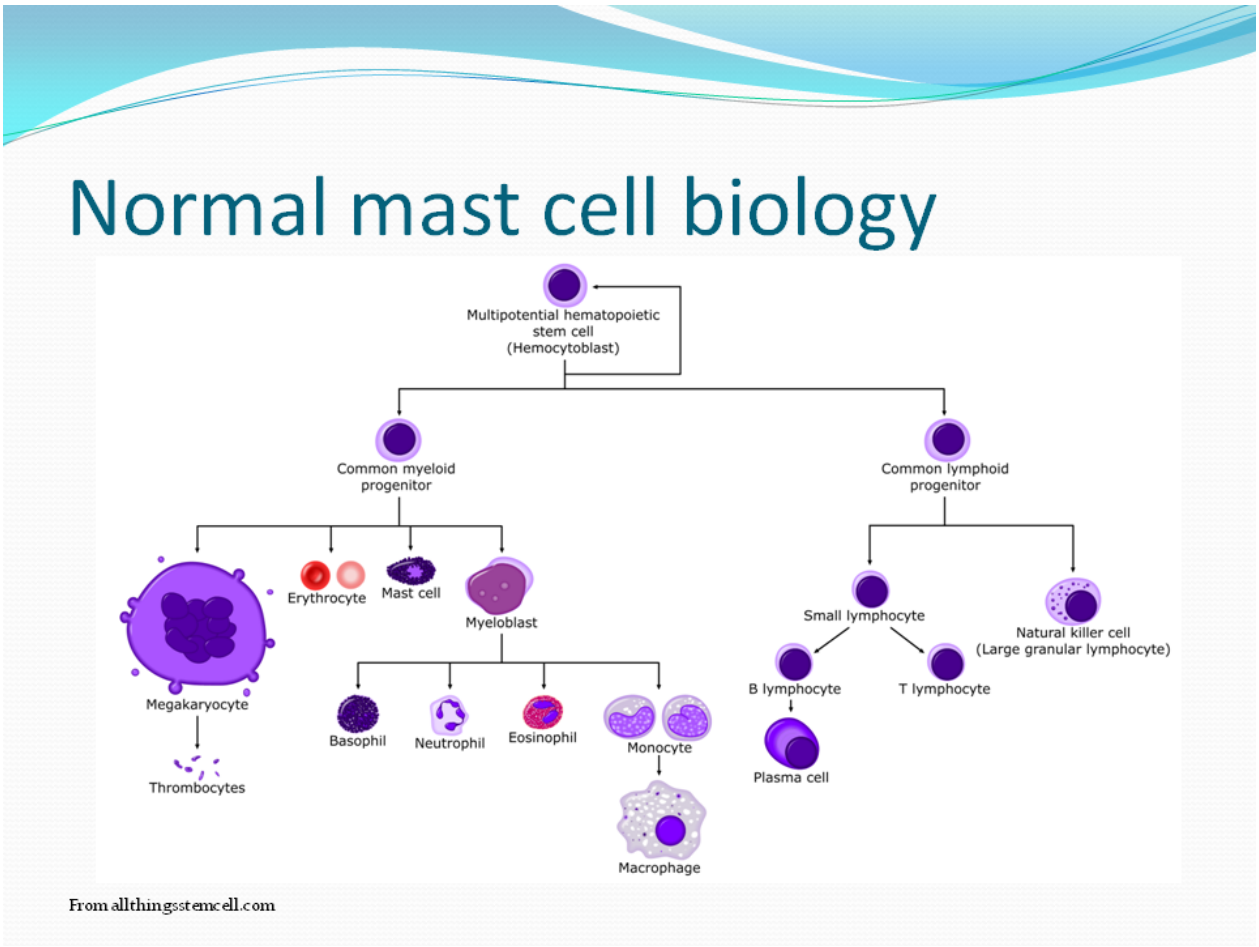
Dr. Werb: Thyroid disease, for example, is so common that 20% of women by the time they're 50 will have something wrong with their thyroid, although it may not need to be treated. So many people with urticaria also have autoimmune thyroid disease. It's not surprising that we resort to assigning the patient the obvious diagnosis even if that usually doesn't explain all the symptoms.

Dr. Monthropey: I was just going to say that during my residency at Sick Kids Hospital, I saw tiny lesions with mastocytosis, but all these were in little kids in whom we never really did systemic workups on them because we were taught that it's usually cutaneous and it tends to stay cutaneous. Is that true?

Dr. Afrin: Cutaneous mastocytosis typically behaves the way you've been taught that cutaneous mastocytosis behaves, including tending to present in childhood rather than adulthood, tending to not evolve into systemic mastocytosis, and not uncommonly

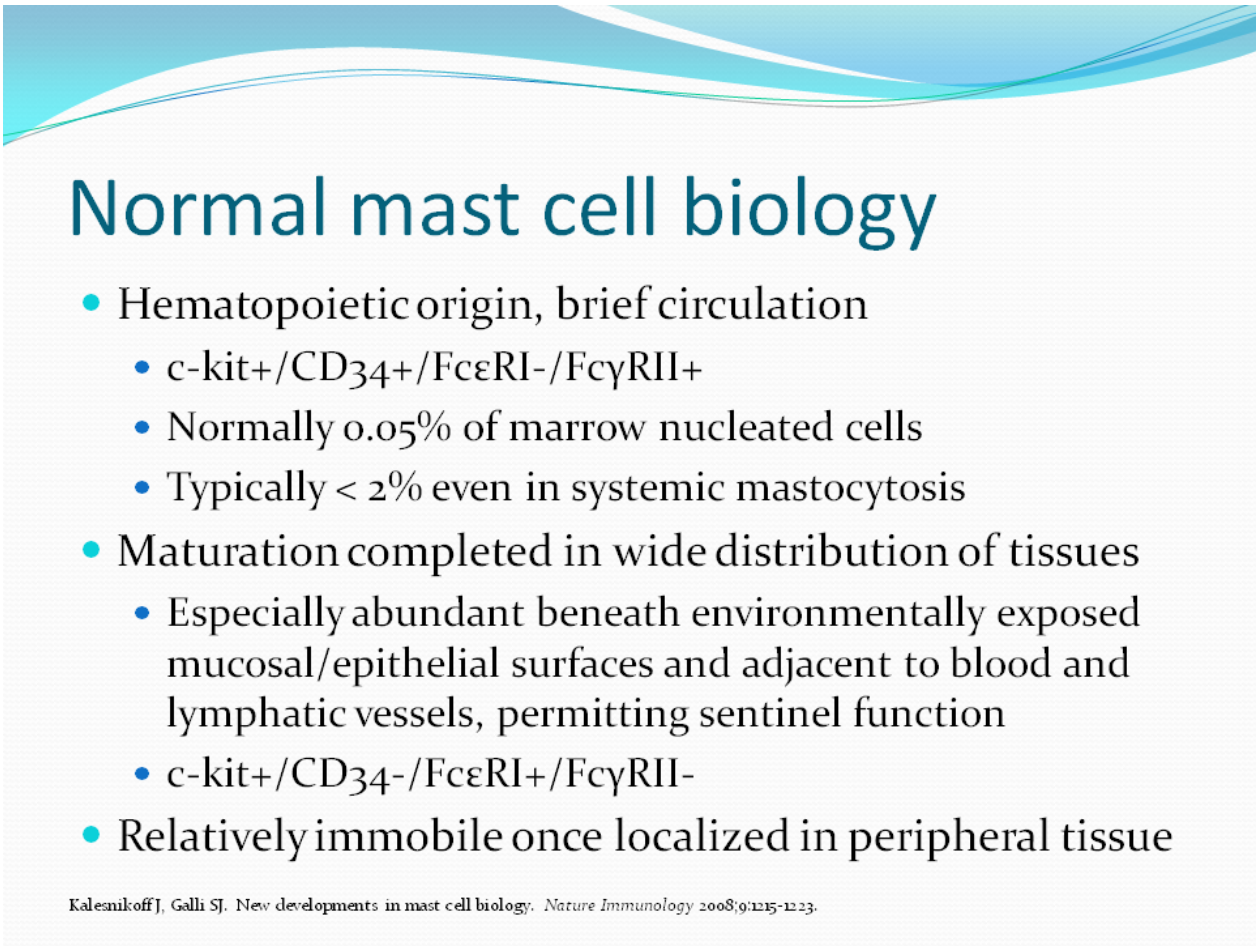
spontaneously resolving in adolescence. Systemic mastocytosis – if it's properly diagnosed according to the WHO criteria – behaves the way you've been taught systemic mastocytosis behaves, but cutaneous and systemic mastocytosis are only a tiny portion of the big picture of mast cell disease, as you'll see better as we keep going through this.

[Slide 23: Normal mast cell biology.]



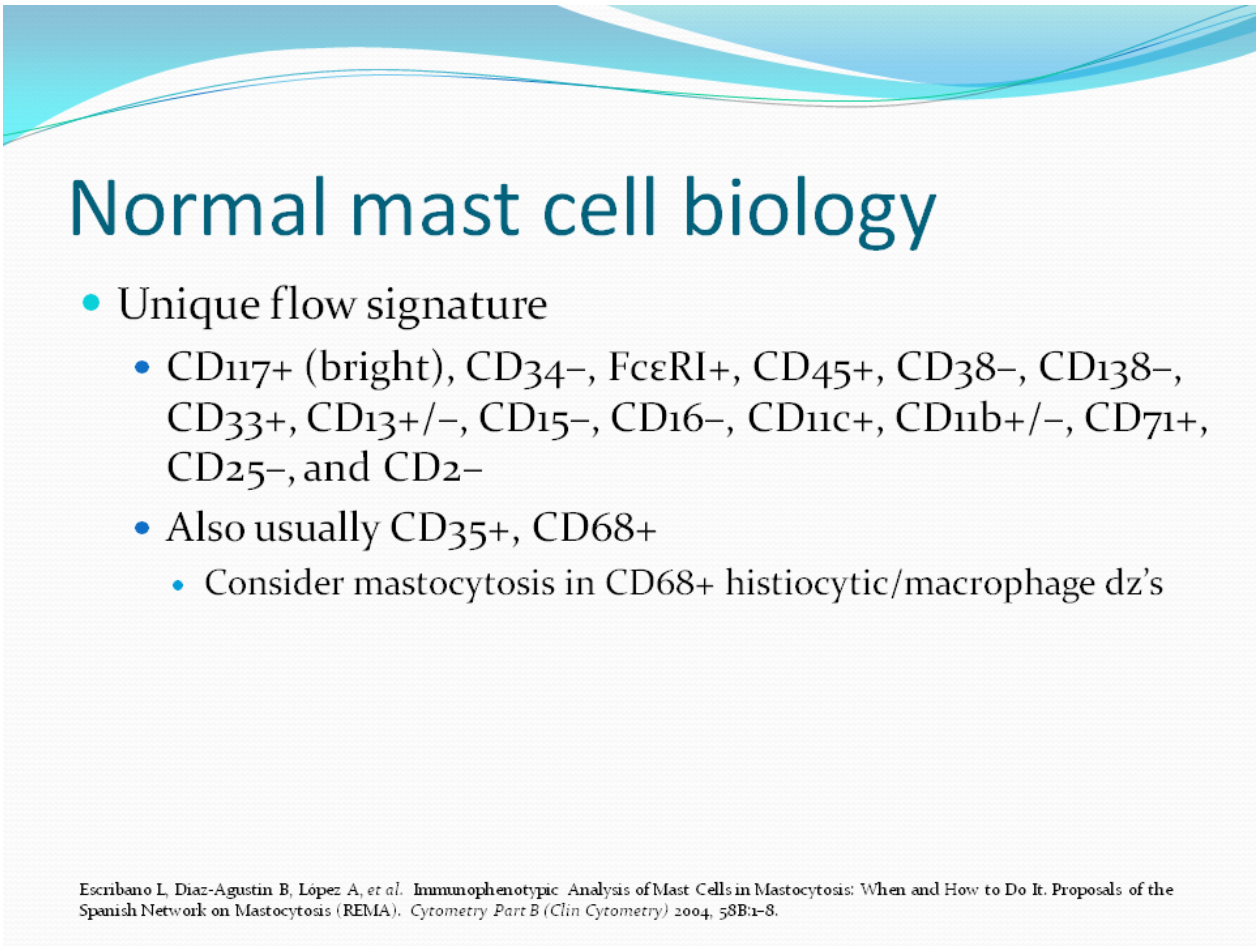
The mast cell is a very primitive cell that differentiates from a common white cell progenitor very early. These are long lived cells that are hard to kill.

[Slide 24: Normal mast cell biology.]



Mast cells are of hematopoietic stem cell origin. They circulate only briefly. They comprise a very small proportion of the marrow cells. They rapidly leave the circulation and enter the peripheral tissues. They are present in every peripheral tissue in the body, where they are relatively immobile and tend to live as long as the patient lives. They are especially abundant at the environmental interfaces, and this is what allows them to perform their evolutionarily critical function as a sentinel. We now believe these may have been some of our first lines of defense to evolve against the toxins in our environment.

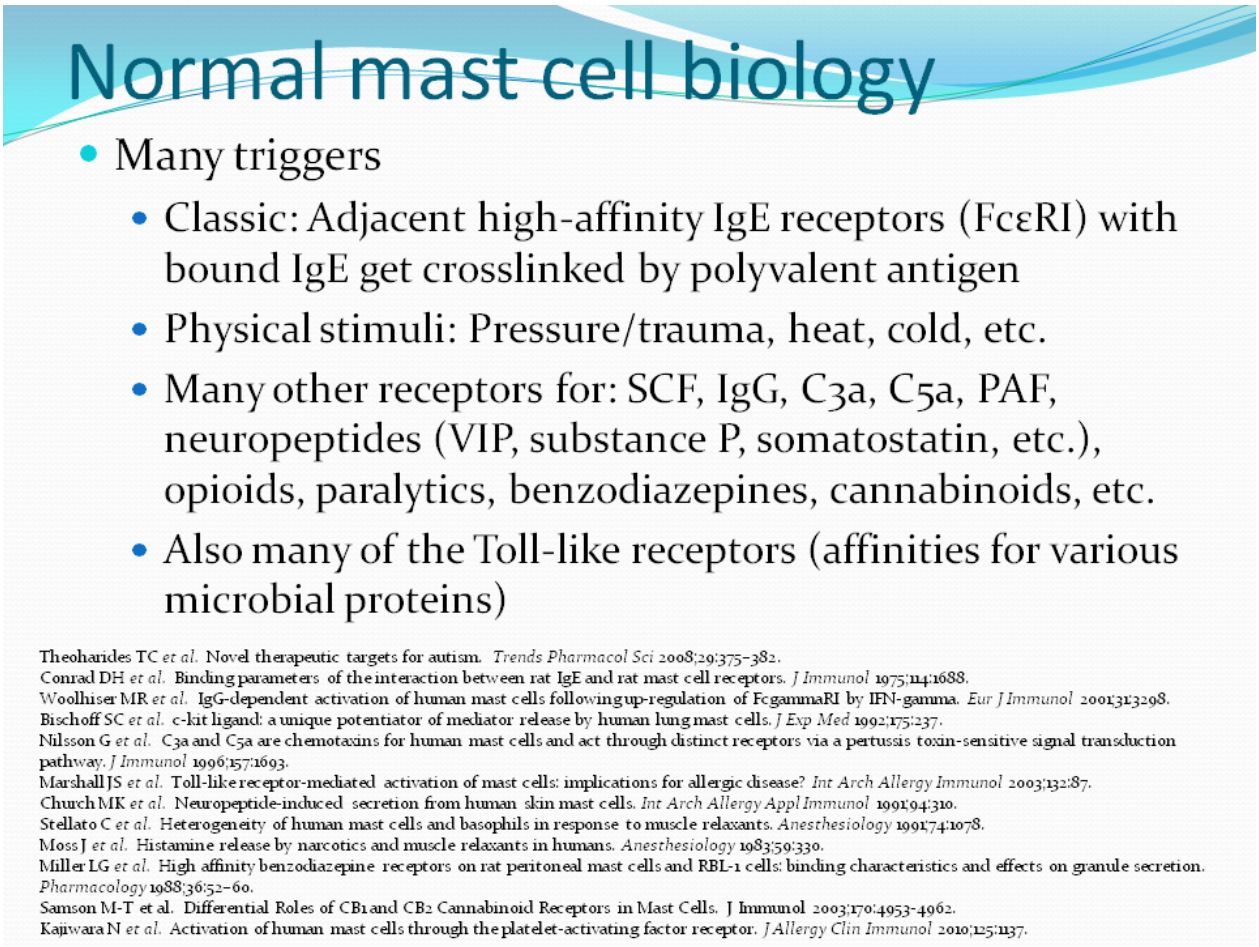
[Slide 25: Normal mast cell biology.]



Mast cells, both normal and abnormal, have a unique flow cytometric signature. These cells are brighter with their cell surface expression of CD117 by an order of magnitude than other cell in the human body. Under normal circumstances, they are always negative for CD25 and CD2. In the abnormal state, they sometimes can co-express CD25 and/or CD2 along with CD117, so if you're going to do a bone marrow biopsy on a patient in whom you clinically suspect mast cell disease, you must send the aspirate for flow cytometry specifically for dual expression of both CD117 and CD25 or CD117 and CD2 and triple expression of CD117, CD25, and CD2.

These cells sometimes are CD68-positive as well. Many pathologists think more about macrophage or histiocyte disease when they see this signature and may not be aware that mast cells can also mark CD68-positive. If you find a patient to have a diagnosis that has been labeled as a histiocytic or macrophage disease, but it's behaving more like a mast cell disease, you may need to go back to that pathology and run the additional testing looking to see if what had been described as macrophages or histiocytes might actually be mast cells.

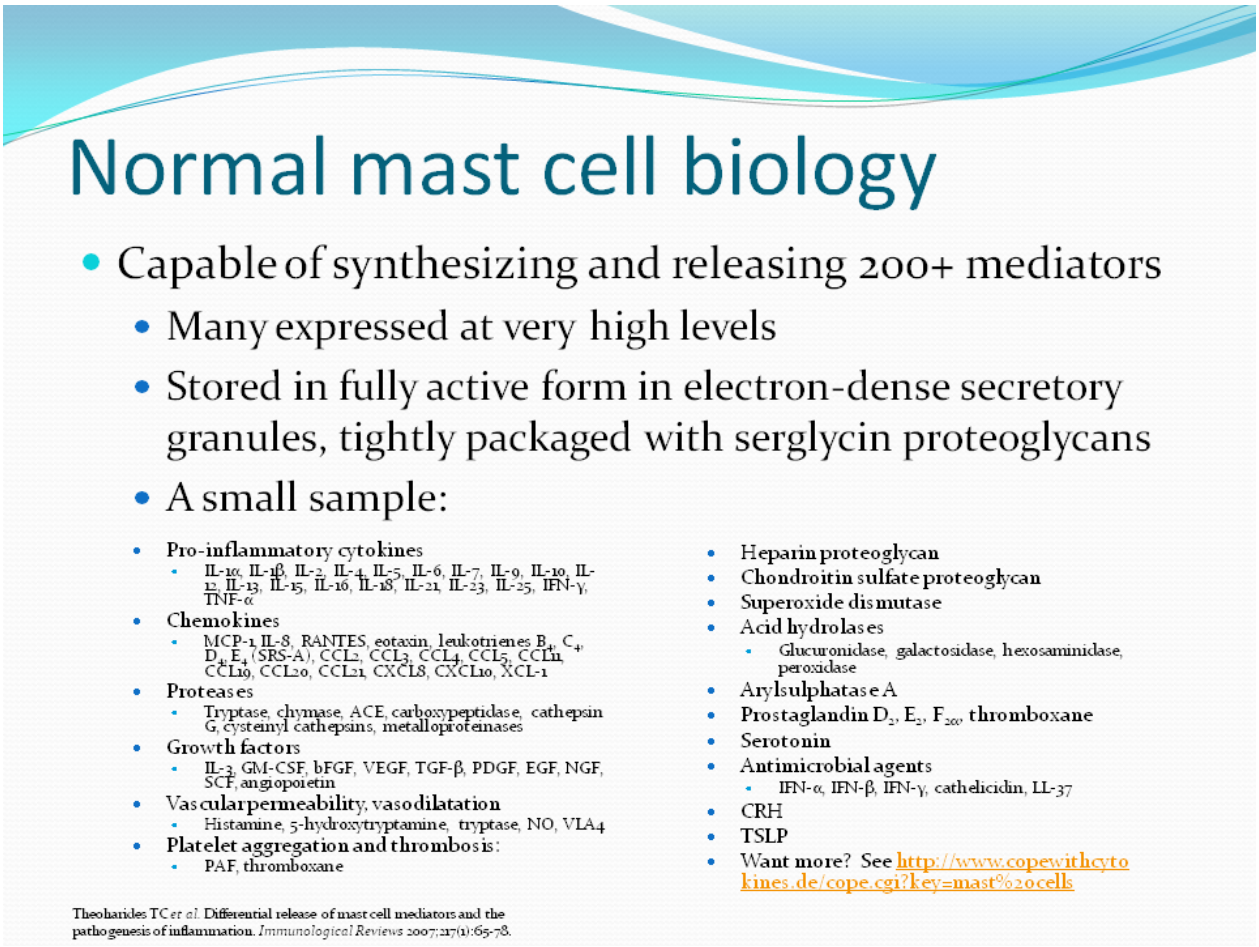
[Slide 26: Normal mast cell biology.]



Mast cells have many triggers, but the classic route to activating a mast cell is mediated through IgE. (I apologize to the non-physicians in the audience that I don't have the time to go into all the details of what that means.) There are many physical stimuli, too, for these cells, such as those listed here, even certain wavelengths of radiation (such as ultraviolet) and electrical stimuli. With virtually every passing month, I am seeing, or hearing about, patients with ever odder physical stimuli for their disease. There are many cell surface receptors beyond just the IgE receptors. Stem cell factor is the ligand for the KIT protein. KIT is the key regulatory gene and protein for the mast cell. We'll get more into that in just a second.

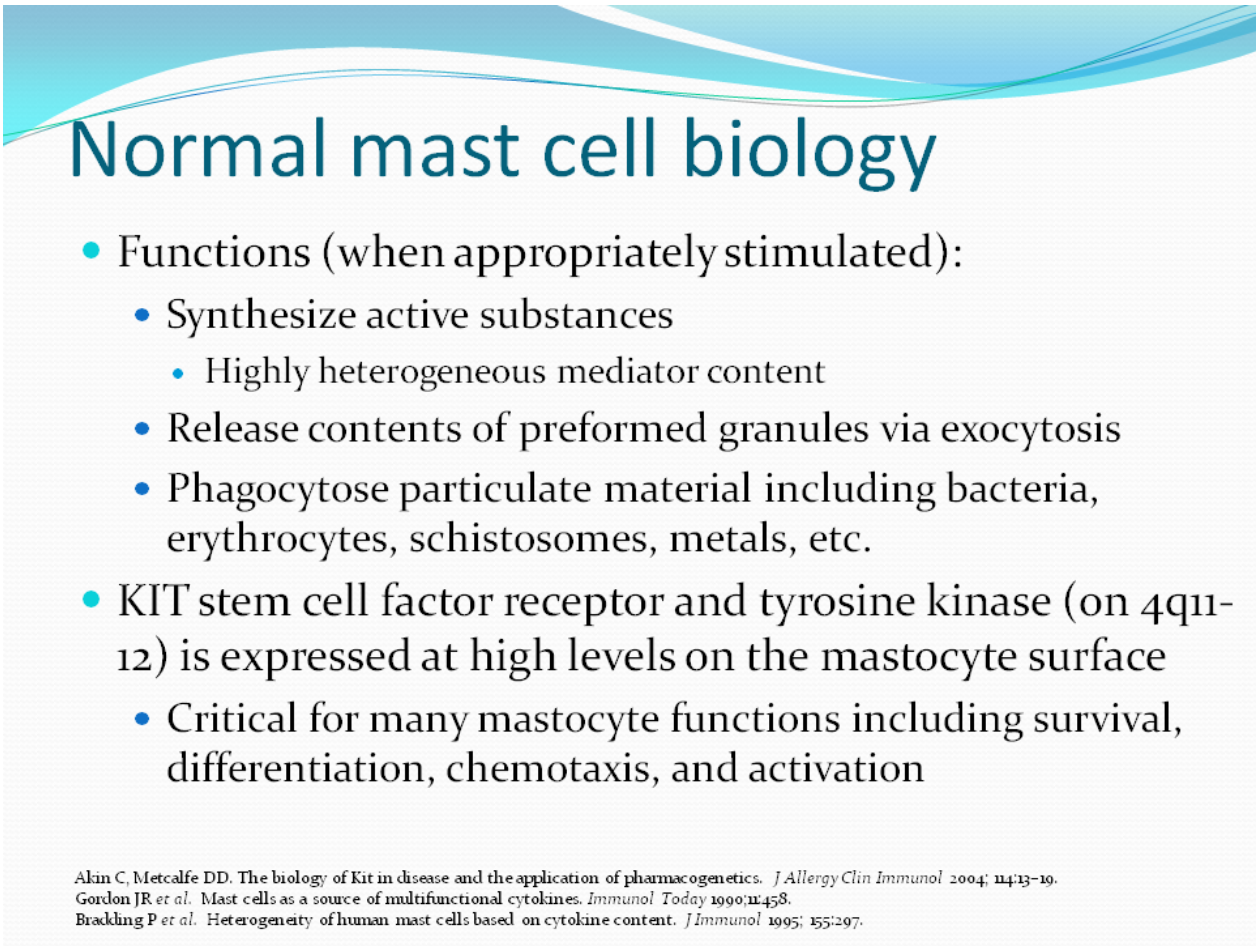
As indicated on this slide, there are lots of other mast cell surface receptors, too, some of which are stimulatory and some of which are inhibitory, all very appropriate given the sentinel function of these cells. These cells have many receptors for different microbial proteins, too.

[Slide 27: Normal mast cell biology.]



These cells are capable of synthesizing and releasing more than two hundred different mediators and receptors, many of which are expressed at very high levels. I know some of the information on this slide is too small to read, but the point of such an extensive listing is not for you to come to know each mediator in detail but rather to appreciate how extensive the mediator list actually is. This list that you see here is very similar to most every mast cell mediator list you will find in textbooks, on the Internet, etc. This list here is a relatively short list, and even this list doesn't begin to give you a full sense of what these cells are capable of doing. I refer interested colleagues and interested patients to a website called "COPE With Cytokines", built and maintained by German biologist Horst Ibelgaufts. Even the COPE list is not a complete list of all known mast cell mediators and receptors, but I refer people interested in this topic to the COPE list because it nevertheless is the "most complete" list I have yet found in one place. Again, I know it's not a complete list, but if you want to go to just one place to get the best visceral impact as to the full scope of what these cells are capable of doing, this is the site to go to, and when you navigate through this site to get to the entry for "mast cells," you'll see a very, very long list of mediators and receptors these cells can express.

[Slide 28: Normal mast cell biology.]



The mast cell functions to synthesize active substances and to release those substances when stimulated. KIT is the receptor for stem cell factor. KIT is a tyrosine kinase that's expressed in very high levels on the mast cell surface, and it is critical for many mast cell functions including survival and activation.

[Slide 29: Normal mast cell biology.]

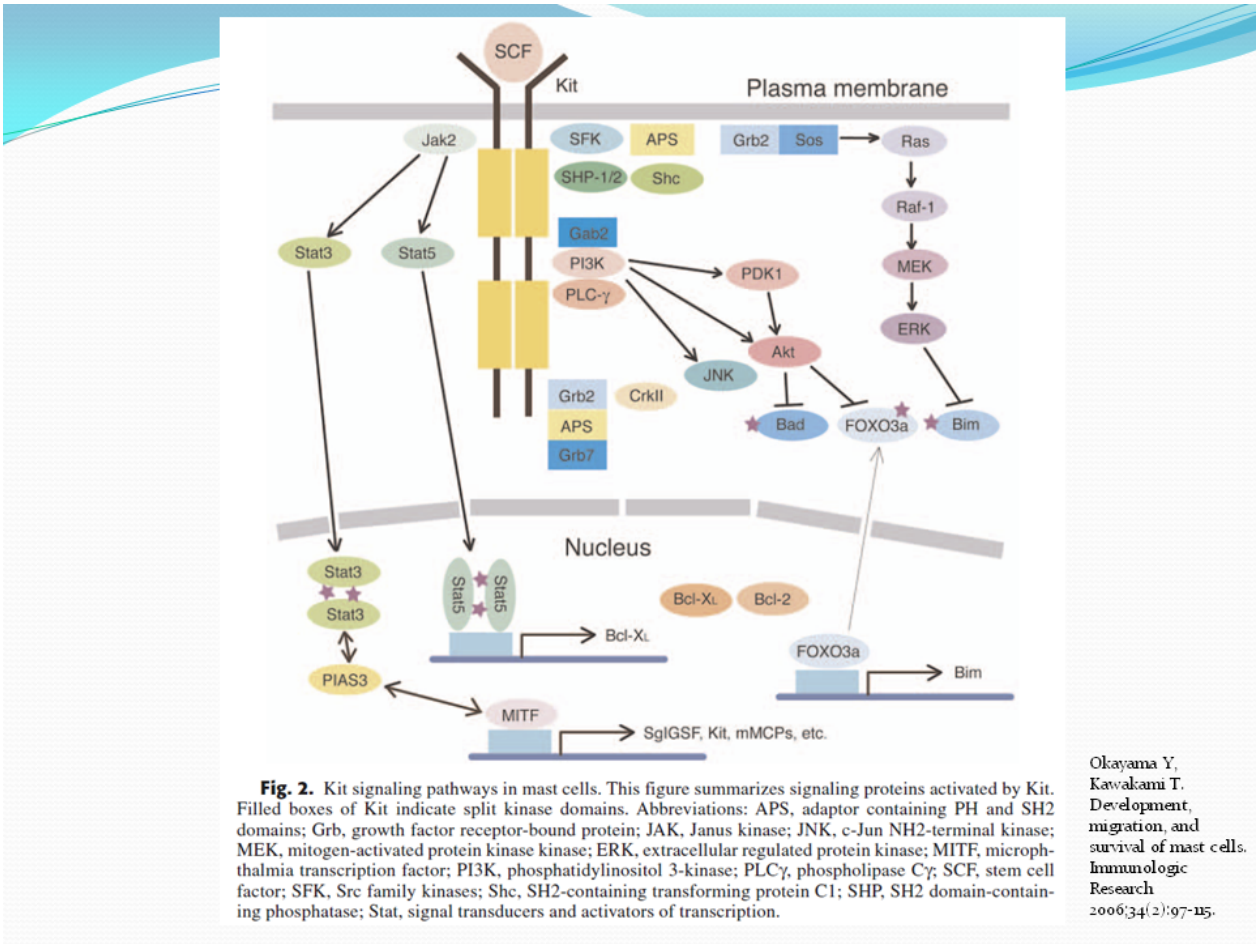


Fig. 2. Kit signaling pathways in mast cells. This figure summarizes signaling proteins activated by Kit. Filled boxes of Kit indicate split kinase domains. Abbreviations: APS, adaptor containing PH and SH2 domains; Grb, growth factor receptor-bound protein; JAK, Janus kinase; JNK, c-Jun NH2-terminal kinase; MEK, mitogen-activated protein kinase kinase; ERK, extracellular regulated protein kinase; MITF, microphthalmia transcription factor; PI3K, phosphatidylinositol 3-kinase; PLCγ, phospholipase Cγ; SCF, stem cell factor; SFK, Src family kinases; Shc, SH2-containing transforming protein C1; SHP, SH2 domain-containing phosphatase; Stat, signal transducers and activators of transcription.

Okayama Y, Kawakami T. Development, migration, and survival of mast cells. Immunologic Research 2006;34(2):97-115.

This is the obligatory molecular pathway diagram in any hematology/oncology presentation. At the top of the diagram, stem cell factor is binding with a homodimer of KIT. This is the normal pathway. Once KIT is activated, that in turn activates multiple downstream pathways, including the JAK-STAT pathway which leads to the production of various mediators which lead to production of many of the constitutional and other symptoms and findings in this disease. JAK-STAT also factors into cell survival and proliferation as well. It also activates the multiple pathways. The point of this slide is that once KIT is activated, there are multiple downstream pathways that get activated.

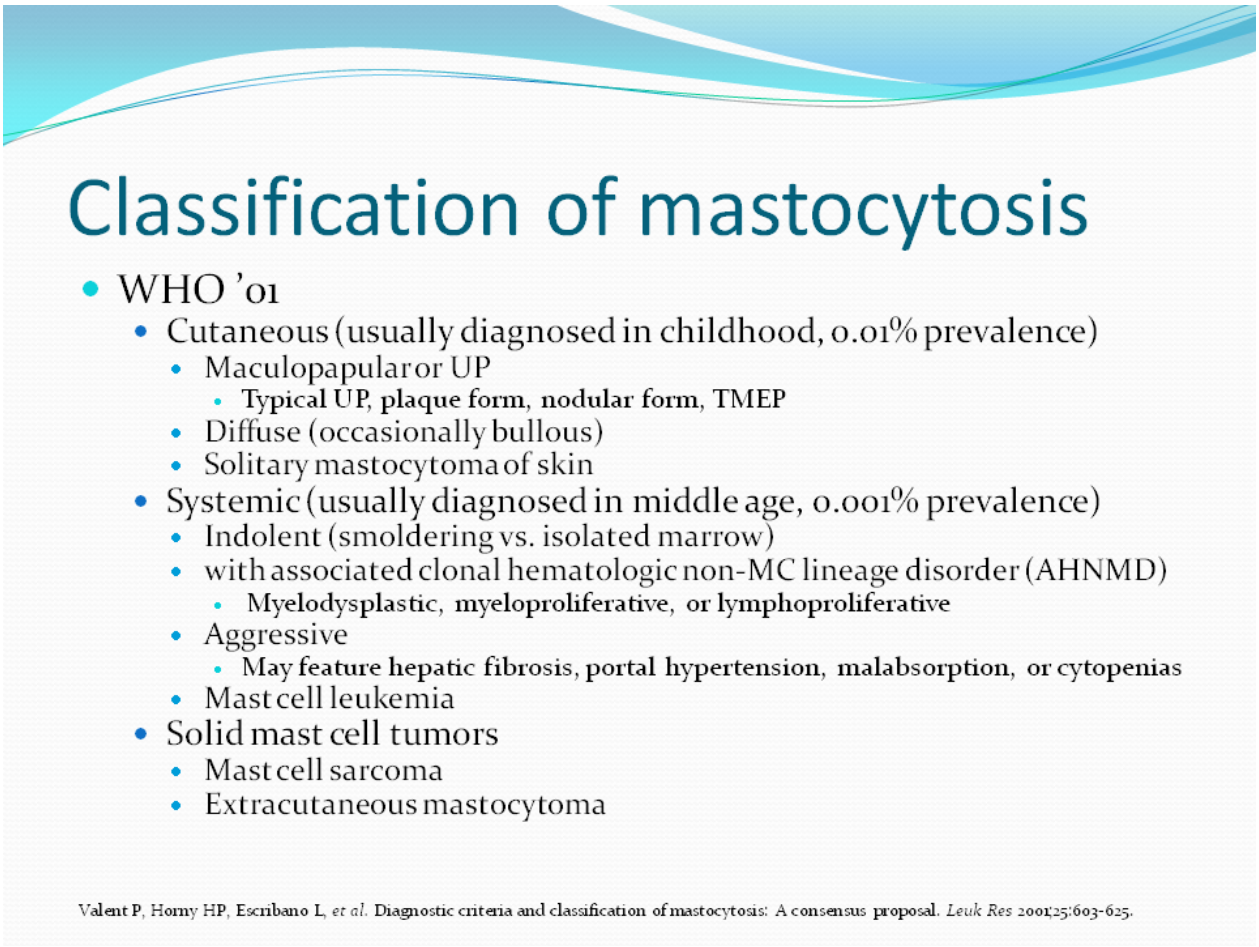
David Girvin: Before you switch slides, on the plethora of triggers that you had, if you're adding triggers to that slide, would it just be an arrow to KIT?

Dr. Afrin: No, because there are plenty of routes for activating mast cells that don't necessarily go through KIT.

David Girvin: Thanks.

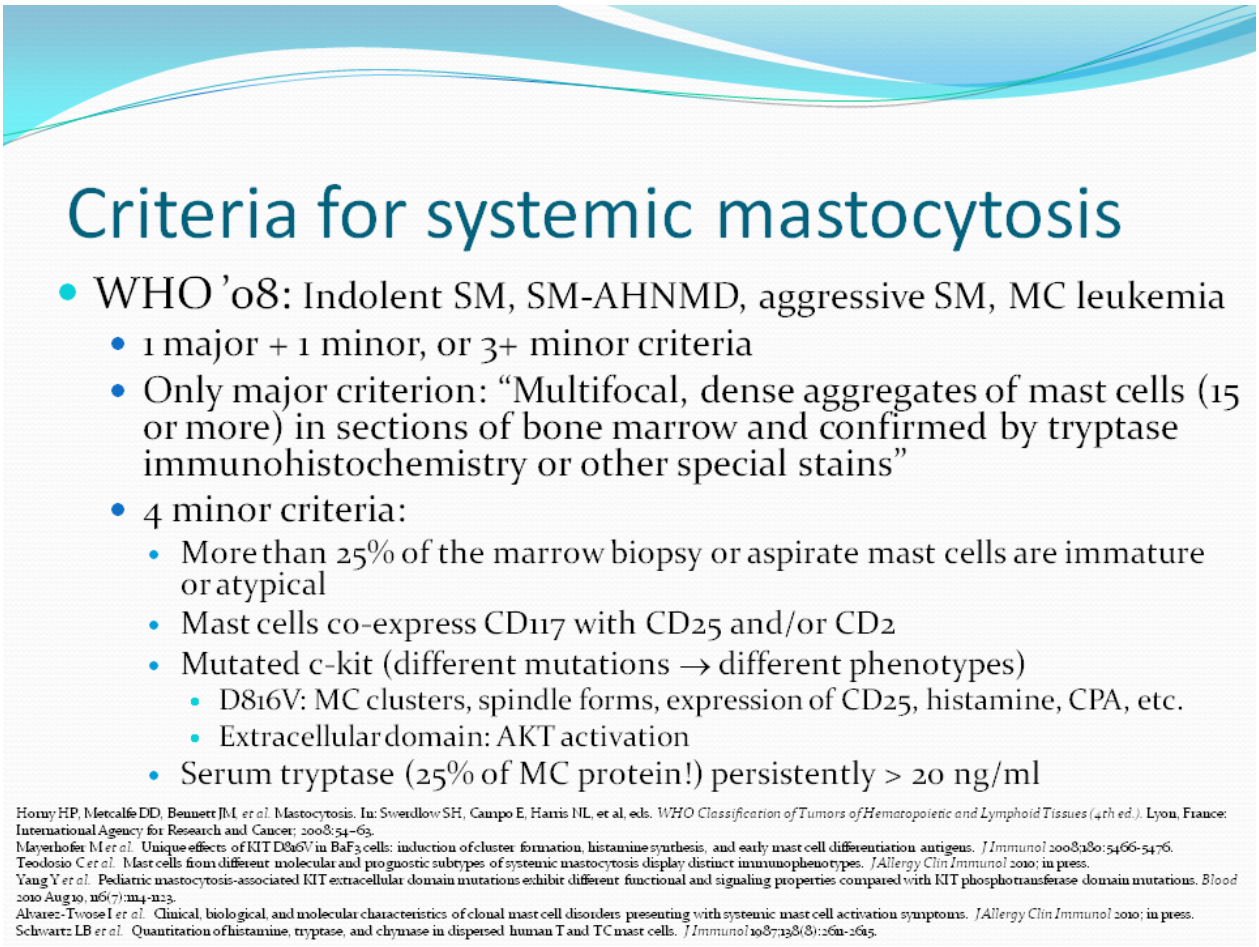
Dr. Afrin: But what's significant….well….hang on just another couple of slides and you'll see something very interesting, where this biology will really come into play.

[Slide 31: Classification of mastocytosis.]



The classification back in 2001 that WHO came up with basically lists 3 forms of mastocytosis – the cutaneous form, which is a rare disease usually diagnosed in childhood; the systemic form, which is an even rarer disease usually diagnosed in middle age, and then the extraordinarily rare solid mast cell tumours (which, curiously, actually are seen not infrequently in dogs and cats).

[Slide 32: Criteria for systemic mastocytosis.]

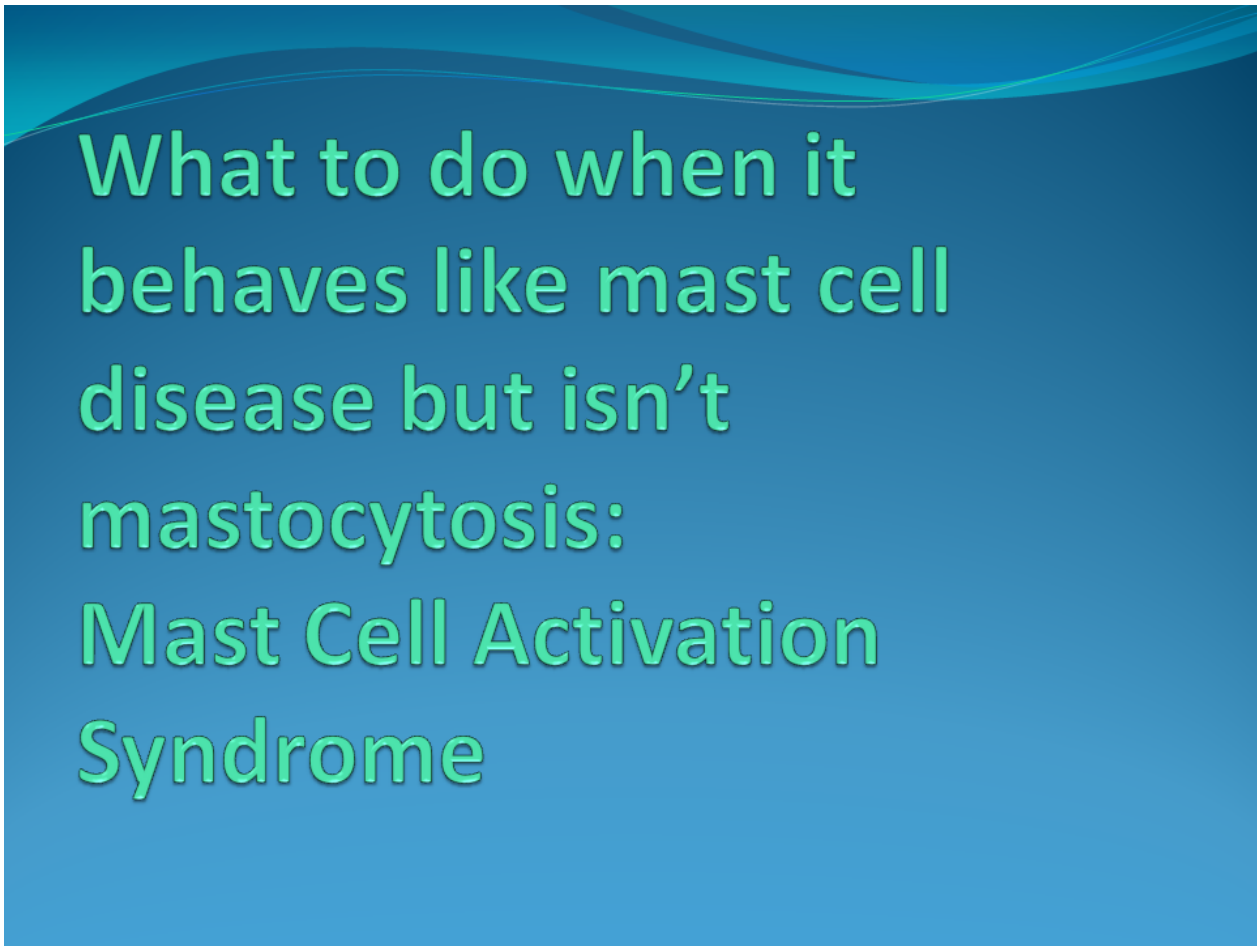


This was updated in 2008. I'm just going to focus on systemic mastocytosis on this slide. First, we have the form of indolent systemic mastocytosis, which is the vast majority of patients with SM. Second, we have the form in which there is a hematologic non-mast cell lineage disorder (which basically means a blood cancer of some sort, like lymphoma or leukemia) associated with the mast cell disease. It is far more often the case that we recognize the lymphoma or leukemia and don't see the underlying mast cell disease unless it happens to be picked up serendipitously in a bone marrow biopsy. Third, aggressive systemic mastocytosis is the form of the disease in which the cells have actually infiltrated into a given organ (for example, the liver) and are causing significant organ dysfunction. And finally, we have the extremely rare – and, for all intents and purposes, universally rapidly fatal – mast cell leukemia.

Now here is where we get into the biggest problem with the WHO criteria. The WHO critieria say that to get diagnosed with systemic mastocytosis, either you must have one major criterion and at least one minor criterion or you must have 3 or more minor criteria. There's only one major criteria: you must have multi-focal aggregates of mast

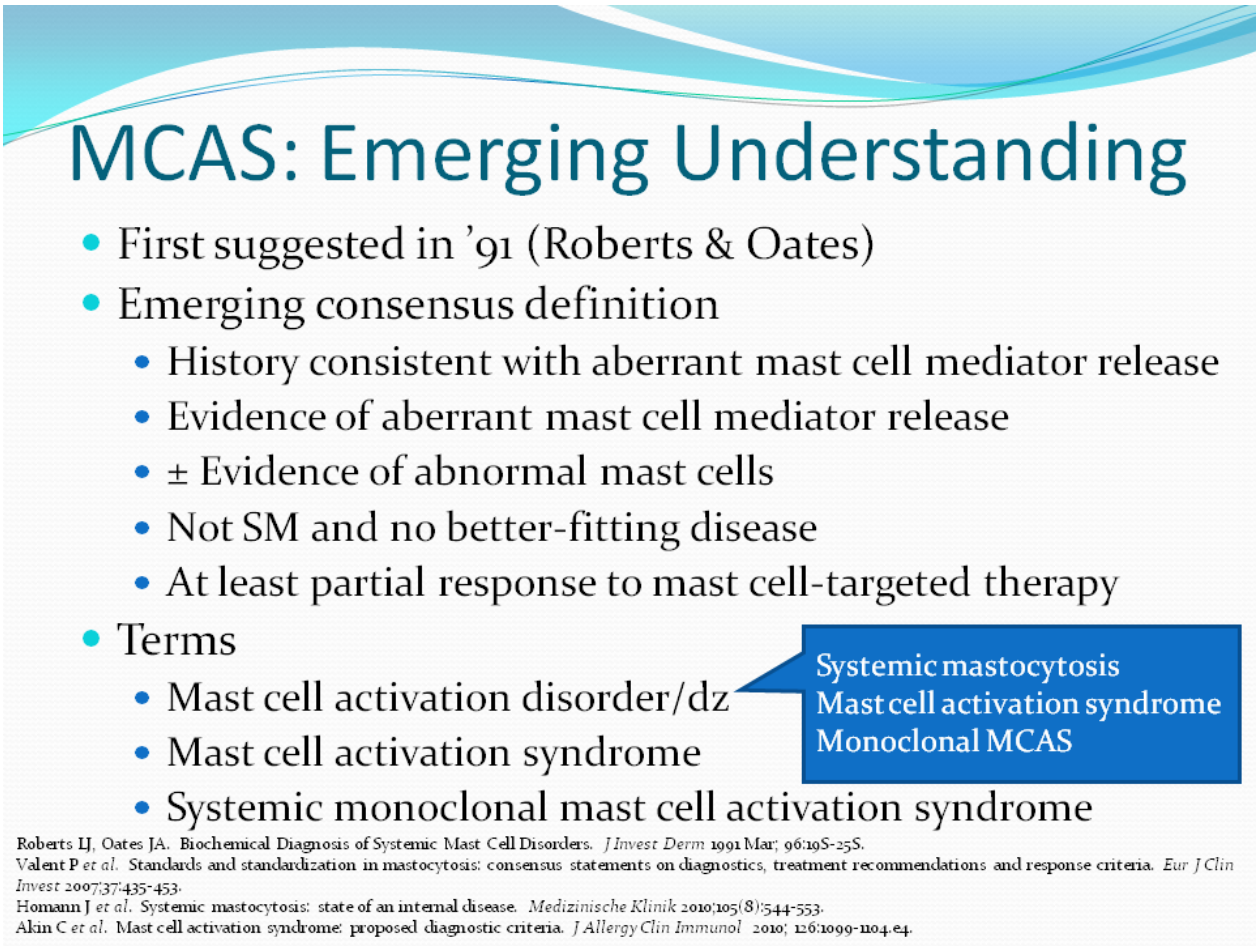
cells in the bone marrow. Not in the gut, not in the skin…and you can't just have diffuse low level infiltration of mast cells in the marrow. There have to be aggregates of 15 mast cells or more. I don't envy the pathologist's having to count these cells in a microscopic field of view. The minor criteria are also listed here: serum tryptase has to be roughly double the upper limit of normal on a persistent basis, or more than 25% of the marrow mast cells have to be atypical, or the pathognomonic flow cytometric signature has to be present, or a KIT mutation has to be proven present, but we have very few KITmutations for which we can commercially test at present.

[Slide 33: The problem.]



So this is the problem: What do you do when it is clinically behaving like systemic mastocytosis, but it doesn't meet the criteria for systemic mastocytosis? For decades now, patients in such a state were denied a diagnosis of not just systemic mastocytosis but mast cell disease in general and often were then discharged by the consultants to whom they were sent for evaluation for mast cell disease. "Sorry, you don't fit the criteria, I don't know what to call you, I don't know what to do with you, and I don't think I can treat you since I can't make a diagnosis since you don't fit the criteria. I'm sorry." But now we do have something we can do for these people. We first of all have a label that we can give them – Mast Cell Activation Syndrome – first suggested back in 1991, as I previously said.

[Slide 34: MCAS: Emerging understanding.]

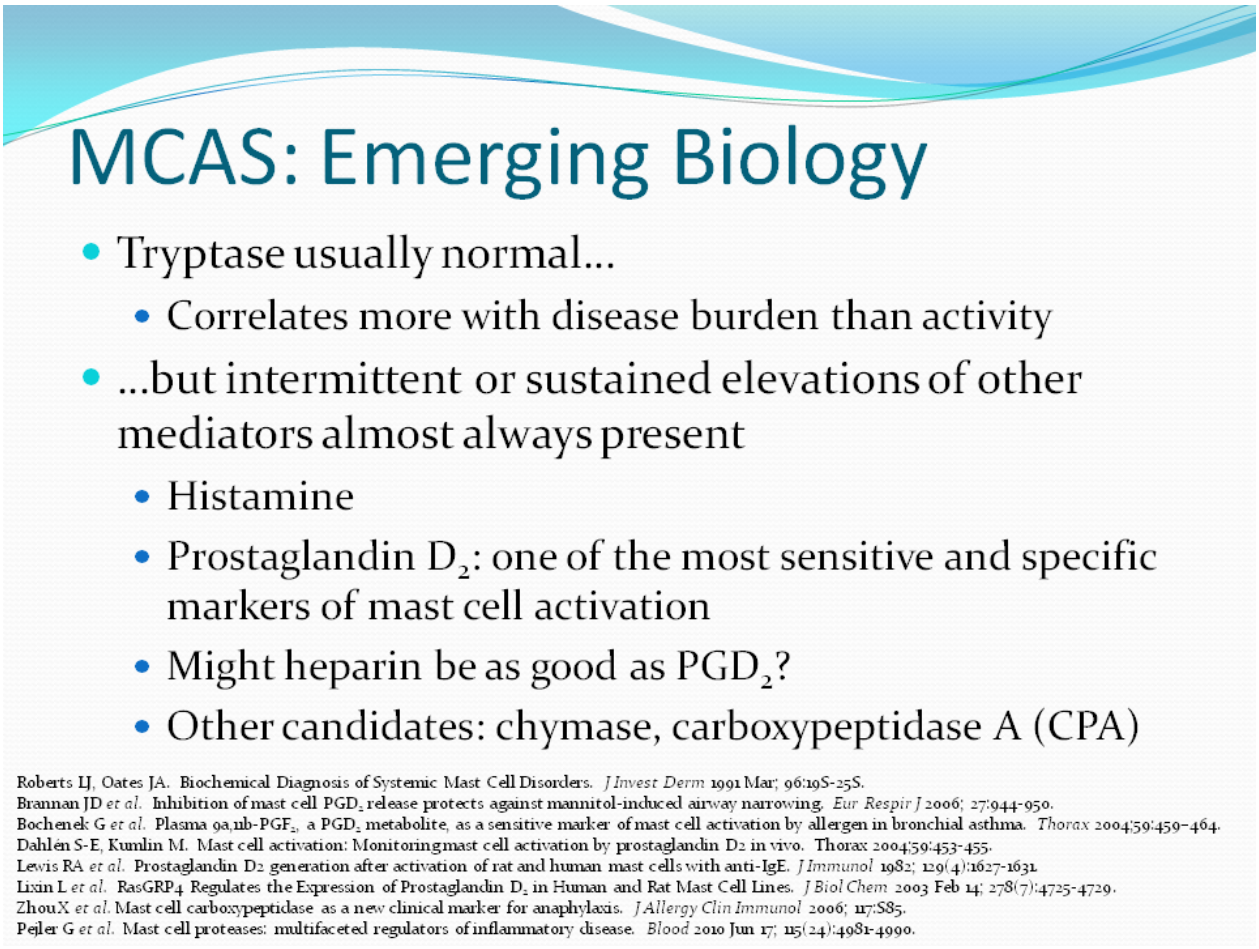


There are multiple competing proposals out there in the literature right now for how to define this. There is not a global consensus definition (for MCAS) out there like there is with the WHO definition for systemic mastocytosis. From reading the different papers on this subject in the literature, though, the emerging consensus for diagnosing MCAS appears to be that you have to (1) have a history that's consistent with the disease (i.e., consistent with symptoms and findings expected from persistent or repeated elevations in mast cell mediator levels), (2) have actual laboratory evidence of aberrant mast cell mediator release, (3) not have systemic mastocytosis, (4) not have evidence of other disease that better explains the full range of findings in the patient, and (5) have at least partial response to mast cell target therapy. This last criterion is the controversial one.

Do you have to actually find the abnormal cells? Some earlier proposals said "Yes," but more recent proposals (such as the one by Dr. Cem Akin and colleagues published in the *Journal of Allergy and Clinical Immunology* in December 2010) say "No" because it's just plain hard to find abnormal mast cells in MCAS and it's generally not "worth it" to find the abnormal mast cells. This disease has been ascribed many different terms by

this point, including mast cell activation disorder or disease, mast cell activation syndrome, and systemic non-clonal mast cell activation syndrome (most recently). ("Non-clonal" may be a misnomer, though, as it only implies that the KIT mutations established in systemic mastocytosis are not present. Whole KIT sequencing, currently not routinely commercially available, is beginning to find frequent mutations in other aspects of KIT.) What has come to pass is that "mast cell activation disorder" or "mast cell activation disease" has come to be the umbrella term encompassing all mast cell diseases.

[Slide 35: MCAS: Emerging biology.]



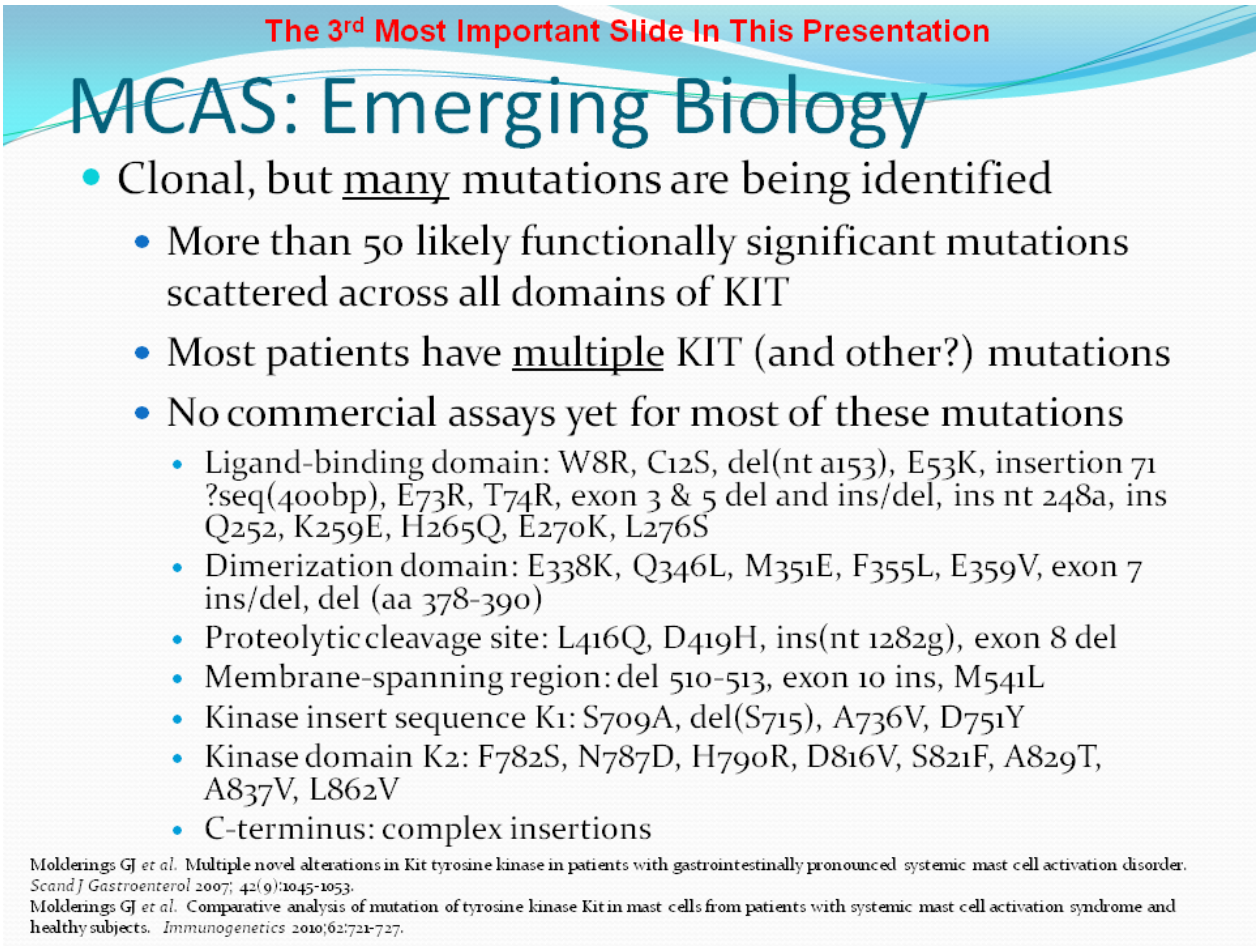
The serum tryptase level is usually normal in MCAS. The level of serum tryptase is now thought by most workers in this field to reflect the total body load of mast cells in most cases of mast cell disease, not the activation state of the mast cell. Thus, since in systemic mastocytosis there is a frank proliferation of mast cells, this is why the serum tryptase level is elevated in more than 80% of cases of systemic mastocytosis. But in MCAS we usually don't see great proliferation, and so the tryptase is typically normal or, in a minority of cases, quite mildly elevated, nowhere close to the cutoff required by the WHO. So tryptase is usually normal in MCAS, but intermittent or sustained elevations in other mediators are almost always present – histamines, PGD_2, even heparin. Dr. Gerhard Molderings at the University of Bonn in Germany, together with his team, has found that plasma heparin levels are abnormal in more than 90% of patients with mast cell disease, and, for the hematologists in the audience, this is even in spite of the patient's partial thromboplastin time almost always being normal. There are reasons for that, but I'll need to have that discussion later with interested parties. There are other potential candidate mediators for indicating that a state of mast cell activation exists – chymase, carboxypeptidase A (CPA), etc. CPA may be even more sensitive and

specific than PGD_2, but there is not presently a commercially available test for CPA. One may become available in about a year or two.

Dr. Sibbald: Does MCAS respond to treatments for systemic mastocytosis?

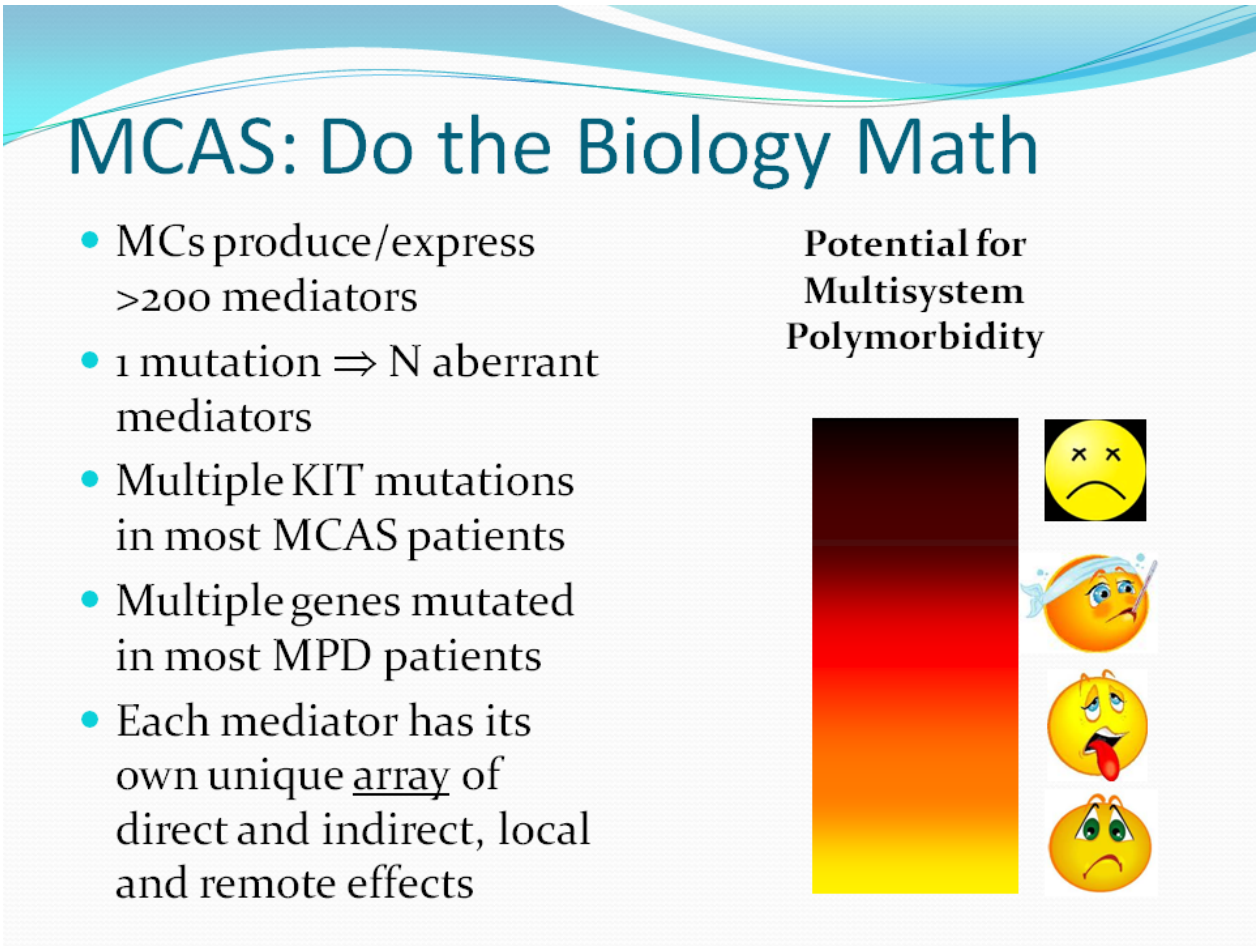
Dr. Afrin: Yes. I'll get into more of that later on.

[Slide 36: MCAS: Emerging biology.]



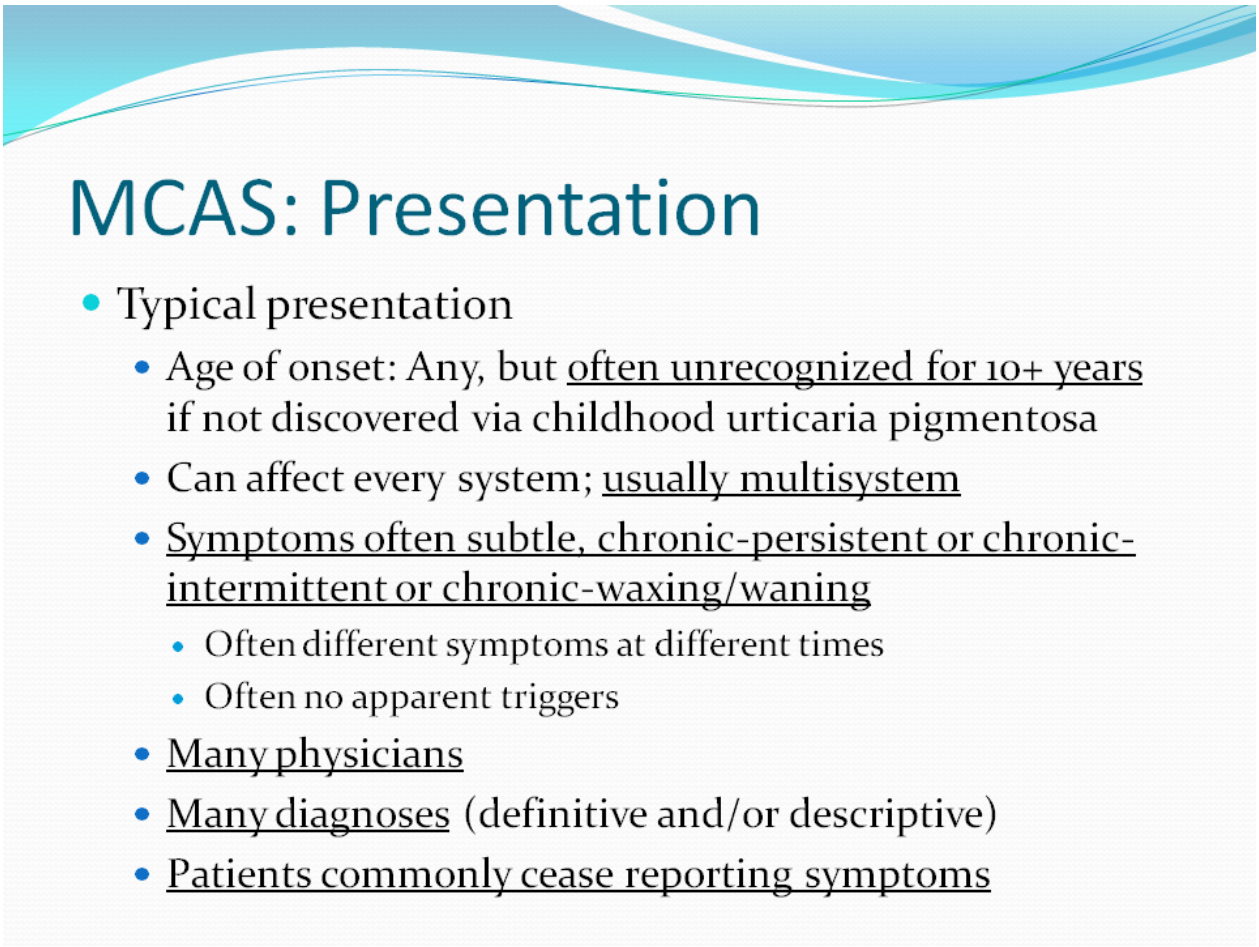
What Dr. Molderings discovered back in 2007, and there was a follow-up paper just last summer (2010) confirming and further extending these findings, is that just as systemic mastocytosis is a clonal disease largely of the D816V mutation in KIT, mast cell activation syndrome is a clonal disease, too, but it is a pool of many different clones. There are many different mutations. Dozens of mutations have been found, and they are scattered across all pieces of KIT [slide 37]. Each mutation leads KIT to behave in a different way, driving the cells to produce different aberrant mediator expression patterns leading to different clinical presentations. And it gets worse (with respect to trying to understand MCAS as a single entity): most patients have multiple KIT mutations, and so far there has been little apparent linkage amongst the different mutations. And then there's another twist that makes the situation worse yet: we've also learned that most of these patients also have mutations in other genes and proteins that are integral to mast cell function.

[Slide 38: MCAS: Do the biology math.]



So, do the biology math. These cells produce and express dozens of mediators. One mutation leads to aberrant expression of maybe not all mediators in the mast cell but probably a sizable subset. But there are multiple mutations in most patients with mast cell disease including mast cell activation syndrome. How any one mutation might interact with any other one or multiple mutations remains to be determined. And there are multiple genes beyond just KIT that are mutated in most myeloproliferative disease patients. And when you go to that "COPE with Cytokines" website, it will really hit this home for you. Each mediator doesn't cause just one problem. Each mediator has an entire array, a unique array, of effects – direct effects, indirect effects, local effects, and remote effects – so when you do all the multiplying implicit in all of this biology math, you begin to understand why this disease could present with such extreme heterogeneity.

[Slide 39: MCAS: Presentation]

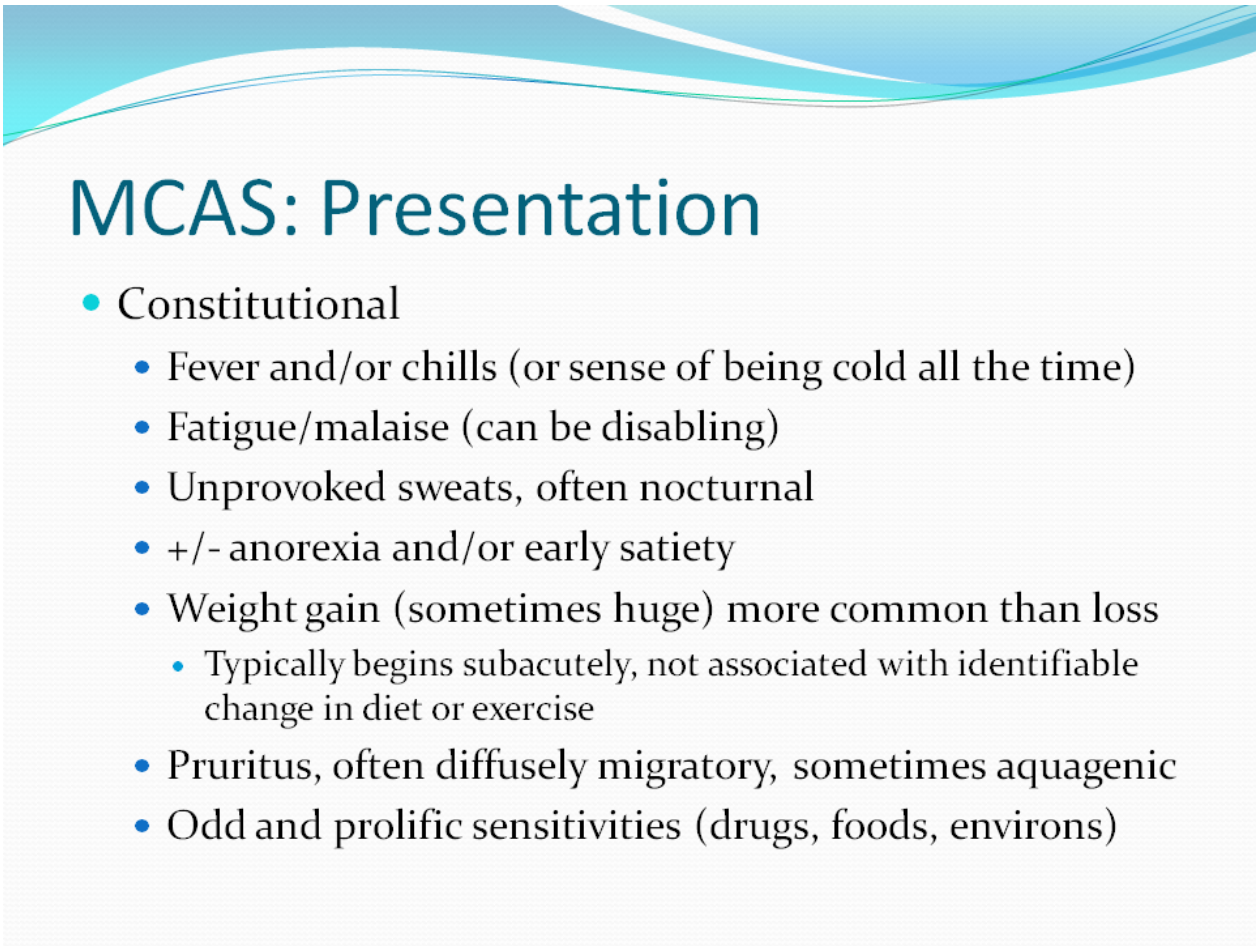


I'm going to go through a number of slides here running down a sort of a review of systems in patients sprinkled with different case presentations.

In the typical presentation, MCAS could be recognized at any age but often goes unrecognized for more than a decade if not discovered in childhood. It's usually multi-system. The symptoms are often subtle, chronic-persistent or chronic-intermittent, or chronic waxing-and-waning. There are different symptoms at different times. Often there are no apparent triggers, but there also are plenty of patients who very clearly know what their triggers are. Be careful, though, to not think that just because the patient says "I get spells for no reason" that it can't be mast cells. Many of them have no apparent triggers. These patients go through doctors by the trainload. They accumulate large problem lists, and after they figure out, after about 2-3 years of symptoms, that their symptoms aren't going to kill them, and they've learned that no doctor is going to be able to figure out a diagnosis, they stop reporting their symptoms. They may well continue to regularly suffer a wide range of symptoms, but they simply stop volunteering such information to the clinicians they see because such abnormality has become their

new normality. So it really is critical, in trying to clinically detect whether mast cell disease might be present, for the clinician to take the time to take a complete review of systems. I have had patients come to me for various hematologic issues, and I start to pick up a flavour that this could be mast cell disease, leading me to run through a long review of symptoms with them (well beyond the symptoms the referral hematologic issue would be expected to produce). Sometimes when I ask, "Do you ever just pass out?," I've had patients tell me, "Oh, yeah, every other day or so for the last 20 years." – and they learned 18 years earlier to stop telling their doctors about it because there was no point to telling their doctors about it. They'd already been through a complete workup and had gotten nowhere and their doctors were getting to the point of just thinking they were crazy.

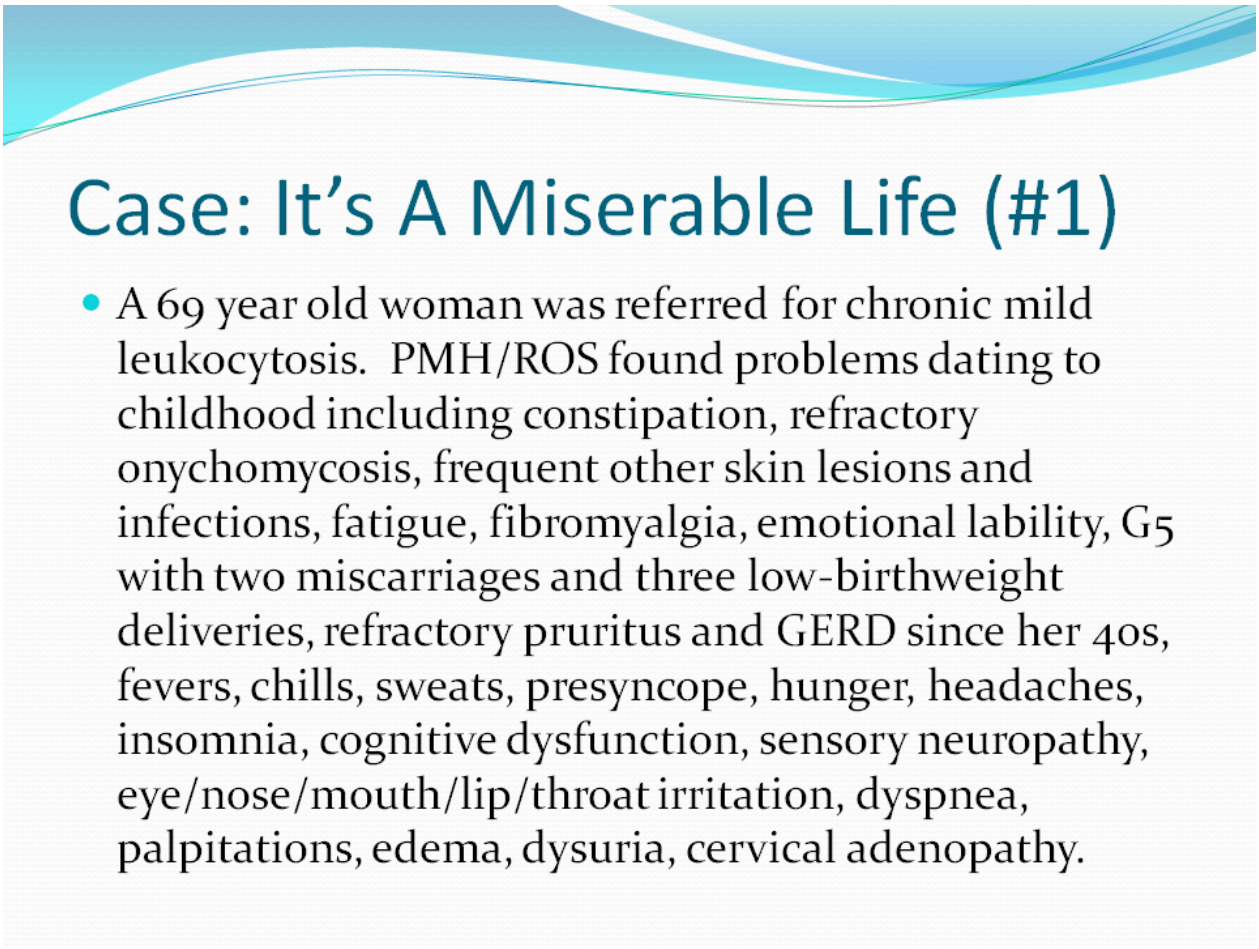
[Slide 40: MCAS: Presentation.]

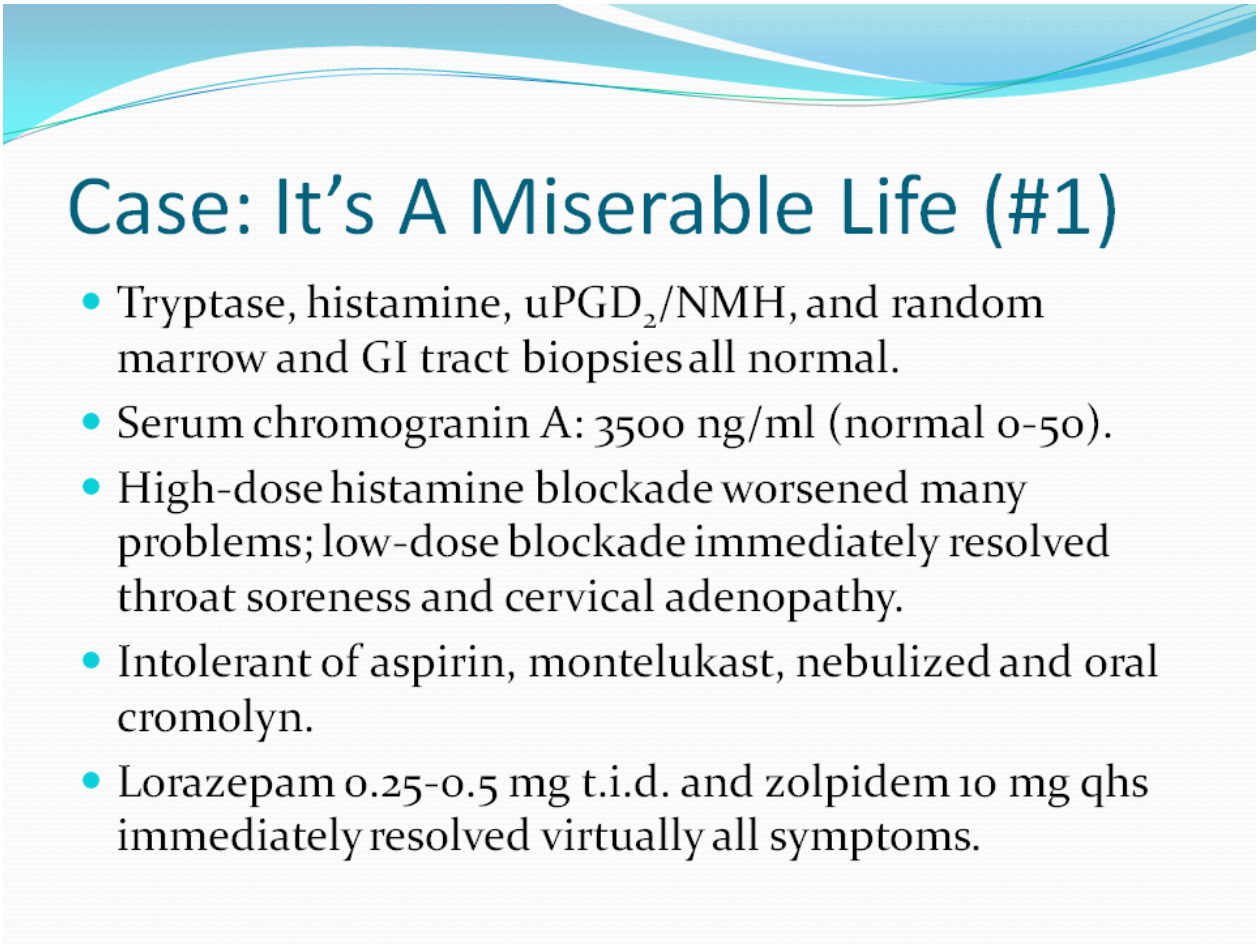


The constitutional issues are all over the map, including fevers and/or chills or, most commonly, neither frank fever nor frank chills but rather just a sense of feeling cold all the time. You have to ask about that specifically because if you just ask about fever and chills, they'll often deny both. The fatigue and malaise can be just terrible, to the point of truly utterly disabling the patient. Unprovoked sweats (often, but not always, isolated to the evening and night hours) are another frequent symptom. Some of these patients have anorexia, weight loss, and sometimes early satiety, usually as a result of an enlarging spleen. Much more commonly, though, we see issues not with weight loss but rather with weight gain, sometimes hugely so. I've had a number of these patients come to me after a gastric bypass which did help them lose weight but didn't address any of the myriad of symptoms they had before the gastric bypass which the surgeon never realized or chose not to address prior to surgery in spite of the much greater likelihood of there being a common problem underlying both the obesity and the plethora of other symptoms.

The pruritis can be anywhere on the scale from non-existent to atrocious. Also, these patients not uncommonly have odd allergies such as things you wouldn't think it's possible to be allergic to such as acetaminophen, levothyroxine, aspirin, loratadine, and so forth. The clinician working in this area also has to keep in mind the possibility that what the patient is reacting to is not the active ingredient but rather a filler, and sometimes simply switching to an alternative formulation of the "same" medication can eliminate or reduce many symptoms.

[Slides 41-42: Case: It's a miserable life (#1).]

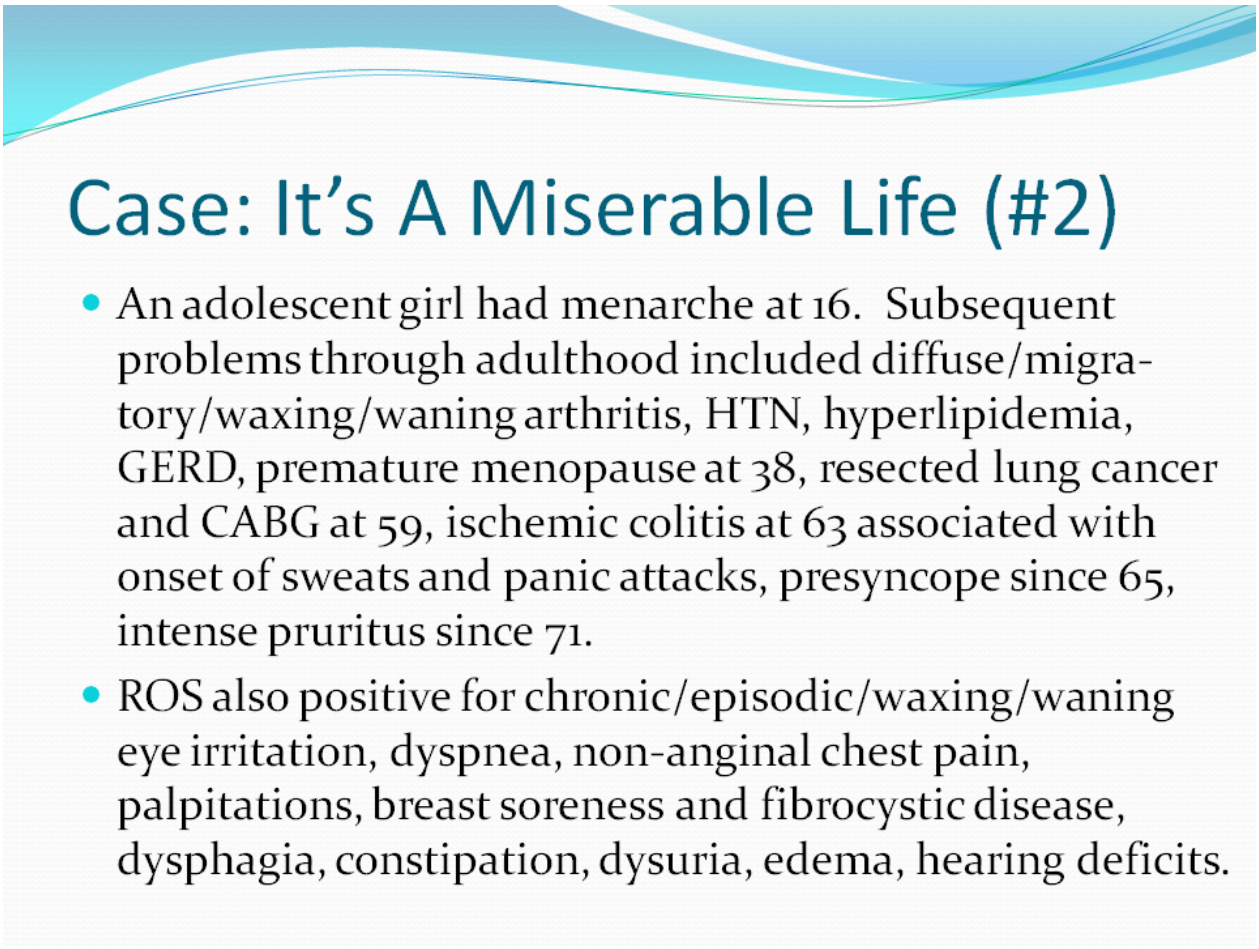


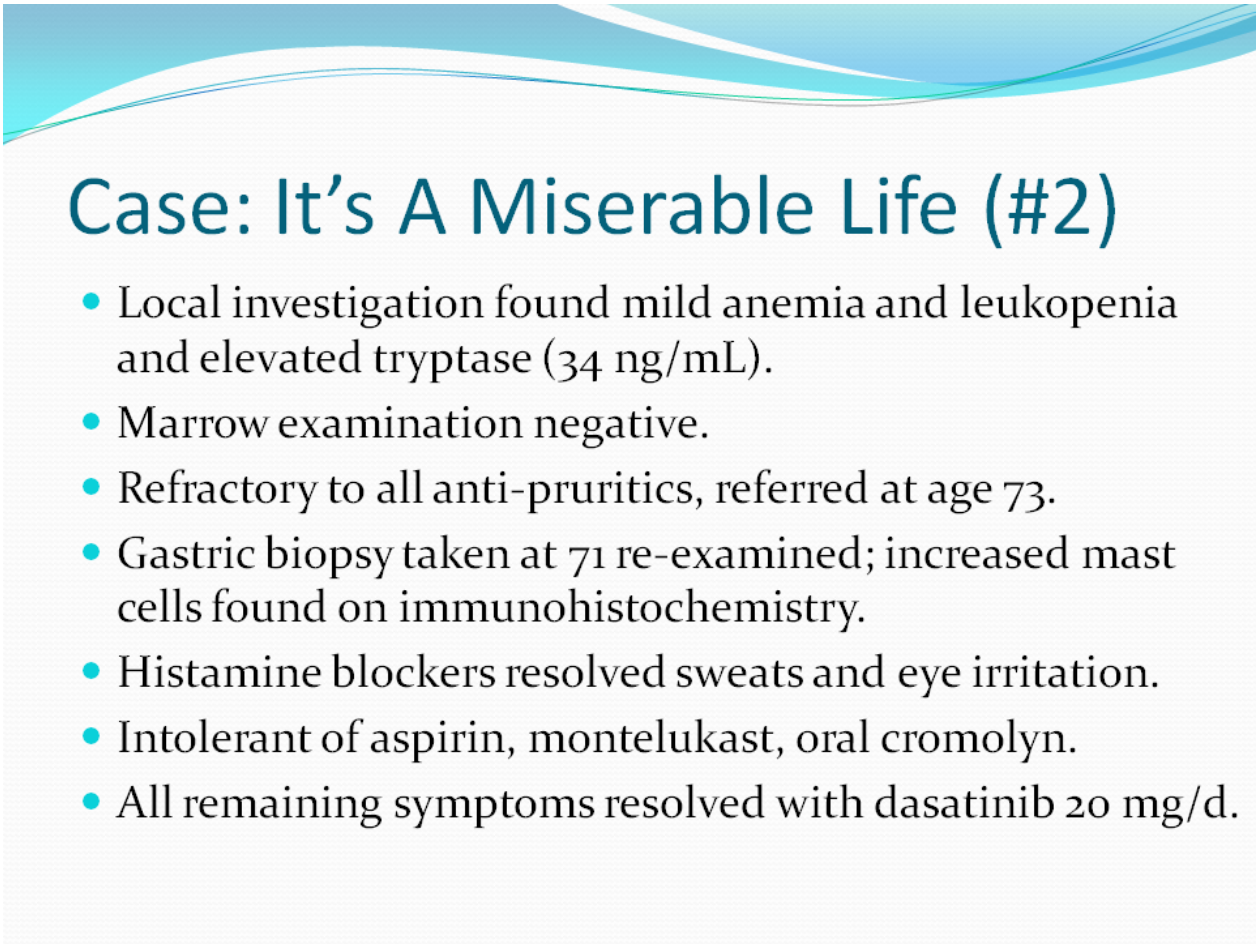


A 69 year old woman was referred for chronic mild elevated white count. I'm not going to go through all the details; suffice to say there was a boatload of problems she had had for decades, going all the way back to childhood. Initial screens for mast cell disease were all negative except her serum chromogranin A level was stratospheric. Most physicians, by their classic training, will tell you a situation like this has to be a neuroendocrine cancer, except cancer patients look and behave in a certain way, and though she was chronically sick to be sure, she didn't look anything like "cancer sick." So there had to be another source for the chromogranin A. Heart and renal failure and proton pump inhibitor use can elevate chromogranin A levels modestly, but nowhere near the levels seen in this patient. Interestingly, when you go digging into the list of mediators the mast cell can elaborate, you quickly come across chromogranin A. So, besides a neuroendocrine cancer that she didn't have and a mast cell disease that fit her chronic multisystem polymorbidity perfectly, there was no other possible source for the chromogranin A. This had to be mast cell disease. High dose histamine blockade curiously worsened many of her problems (perhaps a filler issue?), but the low dose blockade (possibly with a different formulation?) immediately helped in a number of areas. She was intolerant of a number of other cheap treatments, so then we decided to try simple old lorazepam in combination with zolpidem, which is not a benzodiazepine

like lorazepam but nevertheless targets the benzodiazepine receptor. And those two simple little drugs turned off about 95% of her symptoms. At age 69 she was a new woman.

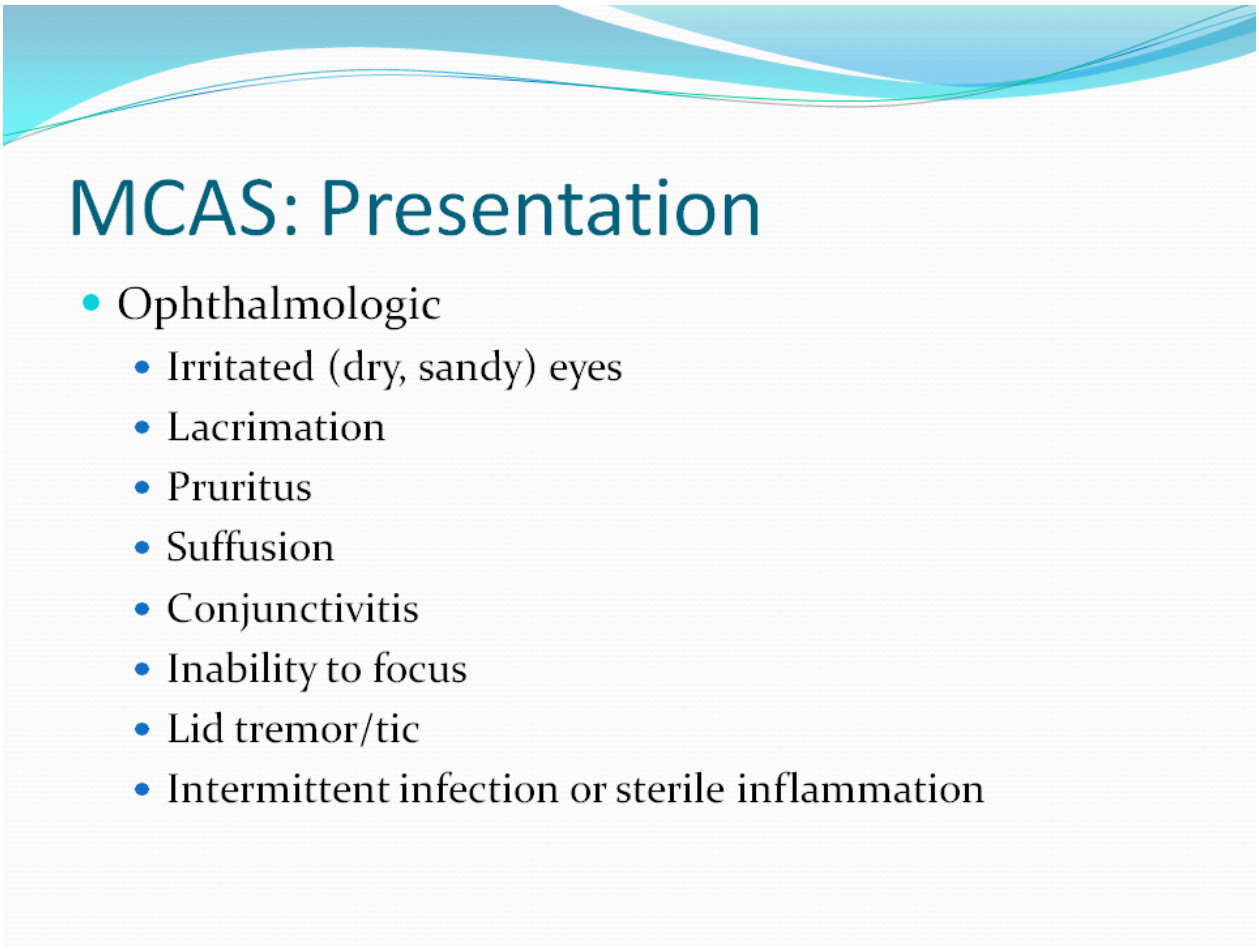
[Slides 43-44: Case: It's a miserable life (#2)]





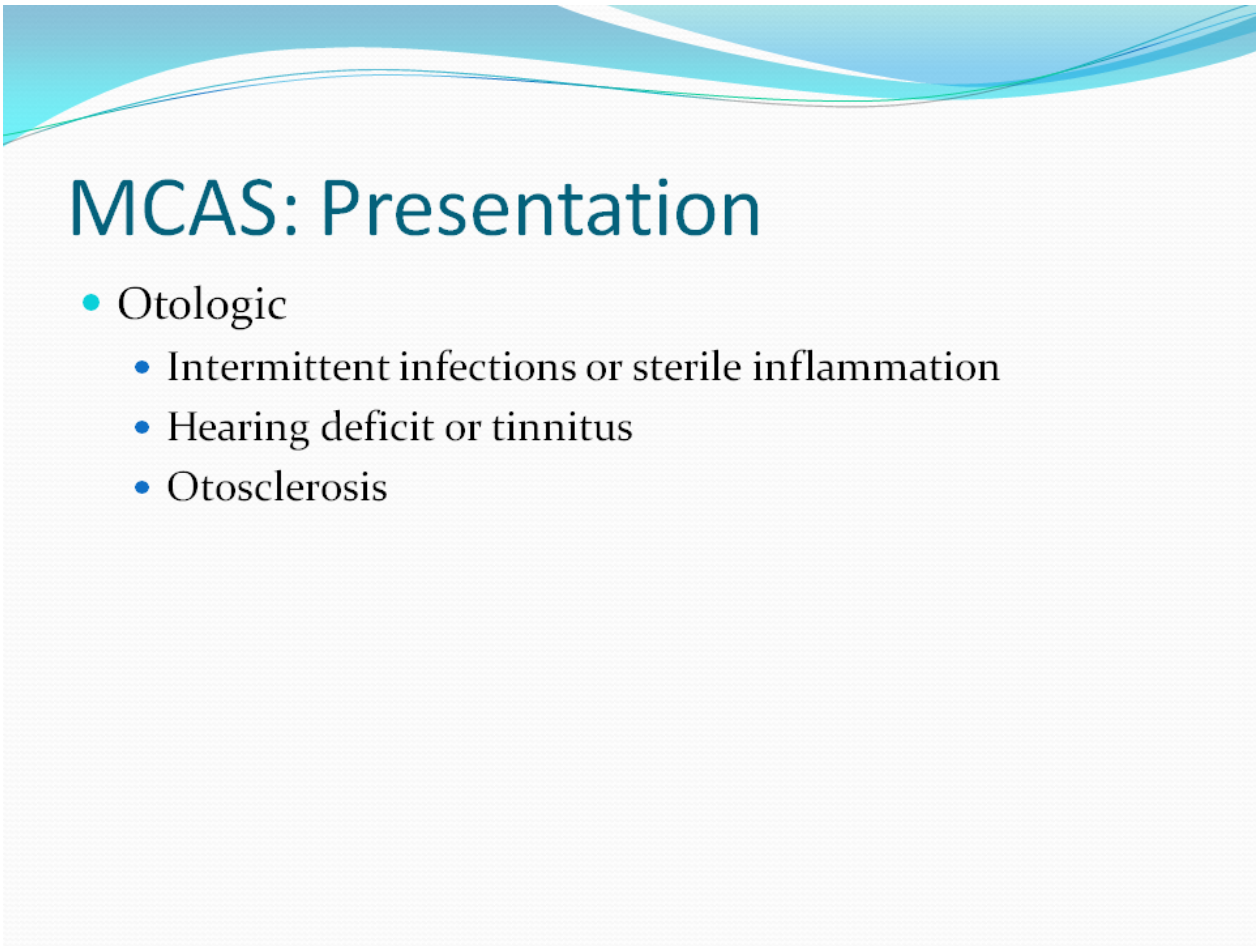
An adolescent girl had delayed menarche at age 16 and many subsequent problems throughout adulthood included not only a first boatload of issues she spontaneously reported when initially seeing me in referral at age 71 but also a second boatload of issues that emerged on a full review of systems. Investigation by her local physicians had found mild anemia, a low white count, and tryptase that was elevated into the range that satisfies the minor diagnostic criterion for systemic mastocytosis, but the marrow was completely negative including the special flow cytometric testing. What do you do? She clearly does not meet the criteria for systemic mastocytosis. She was actually referred to me for refractory severe pruritis. She had been tried on everything. Nothing was helping her with the itching that was destroying her life. I went back to a gastric biopsy she had had done for one of her GI complaints two years earlier, restained it with the right immunohistochemistry – and there was the mast cell disease. Histamine blockers helped only a little bit. She was intolerant of several other drugs. I finally got around to trying her on very-low-dose dasatinib, and it just turned everything off. For her disease it was the right switch, a molecular switch.

[Slide 45: MCAS: Presentation]



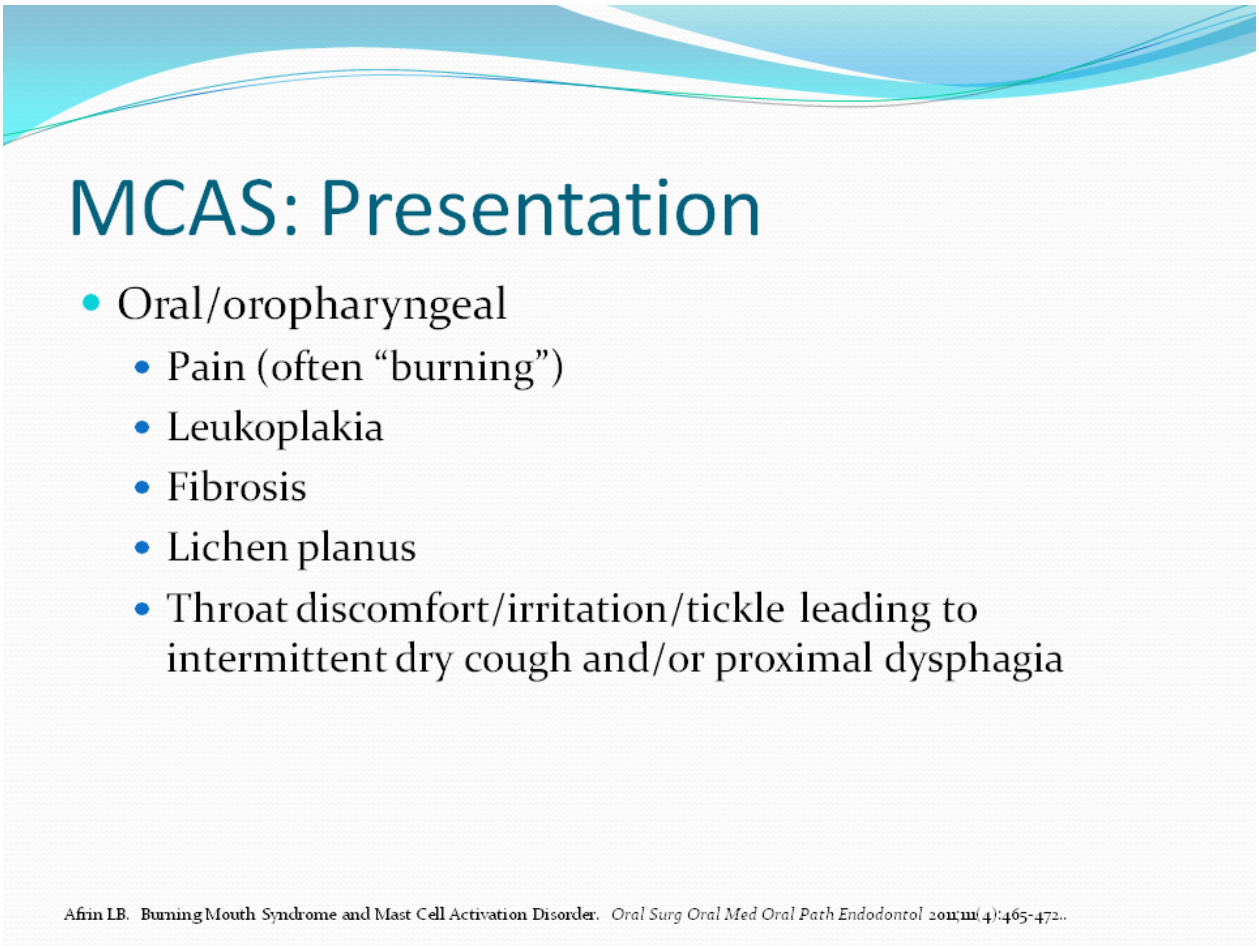
The ophthalmologic issues are all over the map. Irritated eyes, gritty, sandy, dry…you have to use all these words in going through a review of symptoms because they will deny many adjectives but finally acknowledge the one that you hit on. It all boils down to various flavours of inflammation which is usually sterile, but because of the impact the disease has on the immune system, infectious problems can easily arise, so you do have to remain alert to the occasional true infections these patients can have.

[Slide 46: MCAS: Presentation]



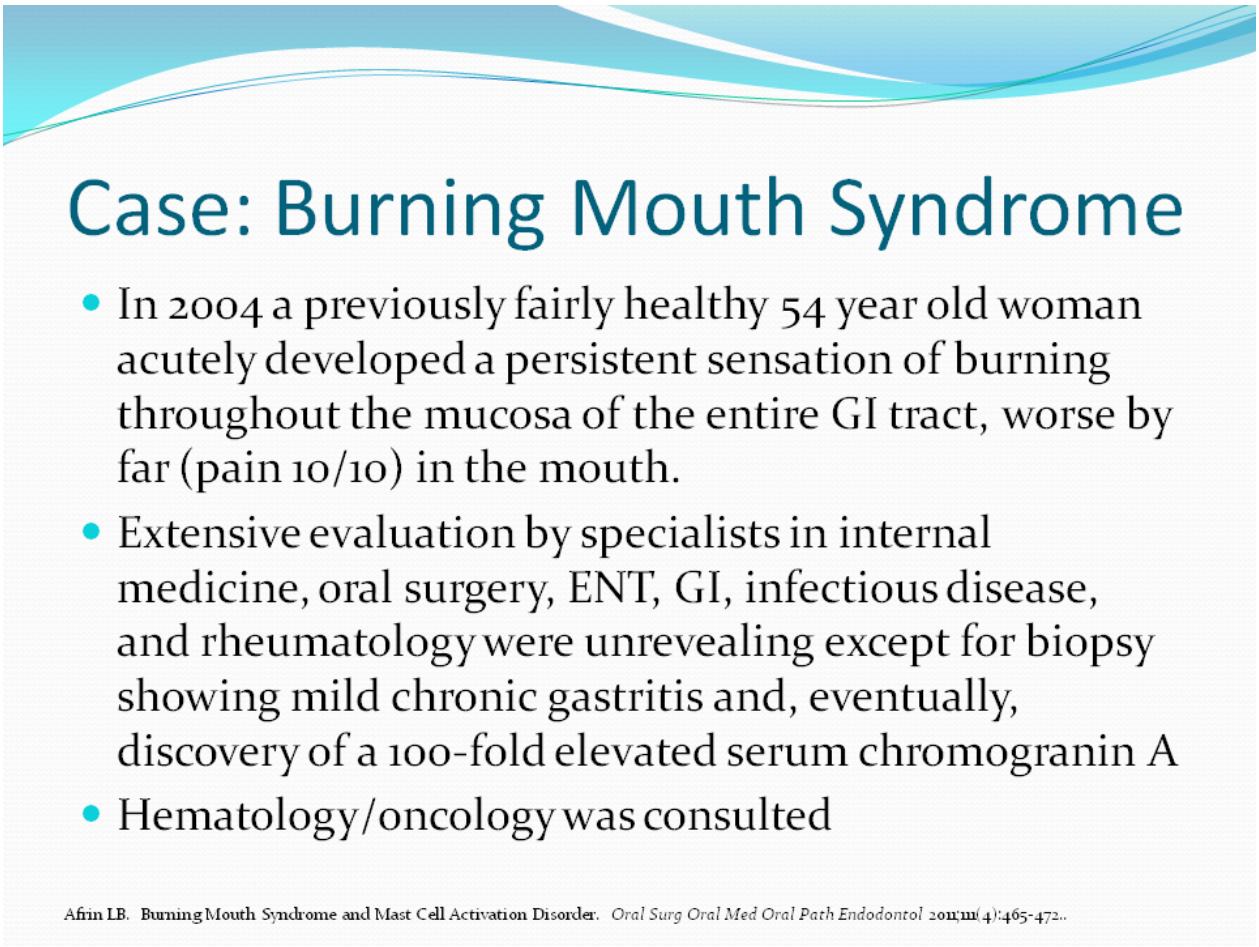
The same sterile or infectious inflammatory issues that arise with the eyes in MCAS can arise in every other system in the body, too, including the ears, where they can present with present waxing and waning tinnitus (a ringing in the ears), hearing deficits, and otosclerosis. I've seen MCAS in several patients now, too, who became idiopathically deaf during childhood or adolescence, and I think I can be forgiven for wondering whether MCAS was the cause of that problem, too.

[Slide 47: MCAS: Presentation]



Oral issues in MCAS are frequent, especially diffuse pain in the mouth that's often described as a burning. They'll deny quite often a soreness or irritation in the throat, but if you ask them, "Do you have this ongoing sense of a need to clear your throat, a tickle in your throat that leads to a chronic cough or a chronic throat clearing?," that's a symptom they will often acknowledge, and it's a low grade inflammation of the pharynx.

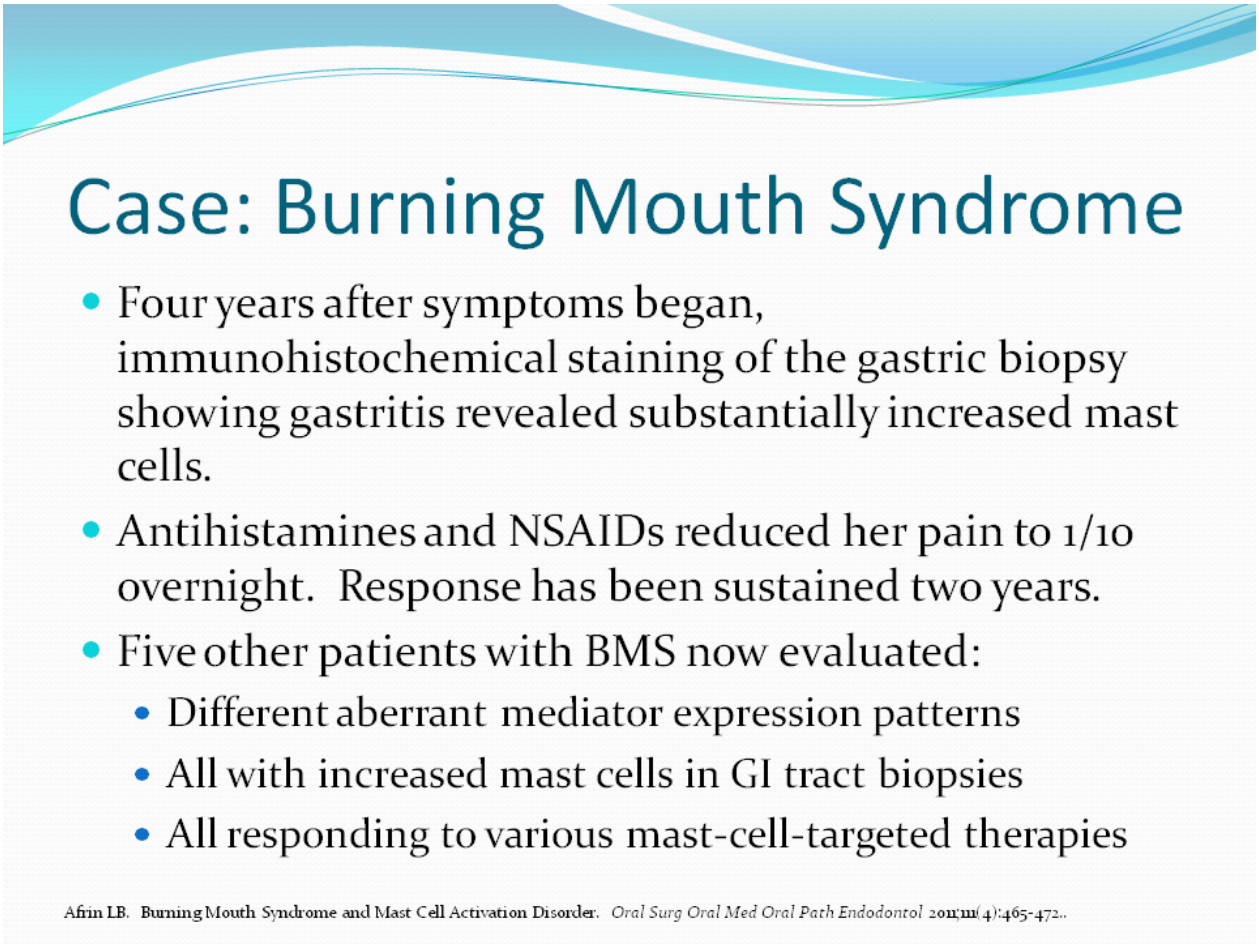
[Slides 48-50: Case: Burning mouth syndrome.]



Case: Burning Mouth Syndrome

- Extensive searching for neuroendocrine cancer (although she didn't look like she had such) was fruitlessly pursued.
- The top five U.S. neuroendocrine cancer experts were consulted by phone; all were absolutely confident she must have a neuroendocrine cancer and recommended periodic repeat testing, especially advanced imaging.
- Evaluation for mast cell disease found negative tryptase and plasma and urinary histamine and PGD_2.
- Two marrow biopsies, two oral mucosa biopsies all nl.

Afrin LB. Burning Mouth Syndrome and Mast Cell Activation Disorder. *Oral Surg Oral Med Oral Path Endodontol* 2011;111(4):465-472..



There actually is a syndrome in the medical literature, primarily in the oral literature, which I certainly never heard about until the middle of the last decade, called burning mouth syndrome. In 1990 a U.S. National Institutes of Health survey found 1% of everybody in the U.S. complains of a chronically burning mouth. Who knew? I didn't. Nevertheless, it's there, and in some people it can be severe enough to require medical attention (though often to little avail).

In 2004 a previously fairly healthy 54 year old woman acutely developed persistent burning throughout the mucosa of the entire GI tract, worse by far in the mouth. She consistently described the pain as 11 out of 10. From the moment she woke up in the morning to the moment she finally exhaustedly fell asleep each night, she was in severe oral pain. Extensive evaluations (including multiple oral mucosa biopsies) were all negative except biopsy of the stomach showed mild chronic gastritis. Multiple types of pain relievers were unhelpful. Nine months after initial onset of her burning mouth pain, she was found by an enterprising gastroenterologist to have a 100-fold elevation in her serum chromogranin A. This is actually the second patient in whom I diagnosed MCAS, just a couple months after my index case of MCAS-induced polycythemia we discussed earlier. The gastroenterologist referred this patient to oncology, saying she had to have

a neuroendocrine cancer. When I initially saw her, she certainly looked chronically ill, but, again, she didn't look "cancer sick." There was just no way she had cancer, yet at that time I didn't know any other disease that could produce a serum chromogranin A level that starkly elevated. So I did my duty as an oncologist and went looking for a neuroendocrine cancer. Starting in 2005 (three years before my index case of MCAS-induced polycythemia), I looked – repeatedly – really hard for her neuroendocrine cancer and just could not find any trace of such a thing beyond the chromogranin level. I called the top 5 neuroendocrine cancer experts in the country, and they all praised how thorough I had been in looking for the neuroendocrine cancer, and they unanimously concluded, "You just have to keep looking because it has to be a neuroendocrine cancer." Around the time I made my index diagnosis of MCAS-induced polycythemia, I began to wonder whether mast cell disease could be causing the burning mouth syndrome here, so I evaluated her for this. It was all negative. Two marrow biopsies and two oral mucosa biopsies, all negative. And then I went back to the old GI biopsies, had the pathologist do the appropriate immunohistochemistry for mast cell disease, and there it was. Antihistamines and non-steroidal anti-inflammatories together reduced her pain overnight from 11/10 to 1/10. She has done well for the last two years. I have now found MCAS underlying burning mouth syndrome in several other patients, and in April 2011 I published in one of the oral medicine journals my first 3 cases of this. Since that was written, though, I've now found another 3 patients with this. I'm not trying to say that all cases of burning mouth syndrome are actually MCAS. There are cases of burning mouth syndrome that are caused by Sjögren's disease, candidiasis, or other causes, but there's an awfully large fraction where the cause is utterly unknown and every study imaginable of the mouth finds nothing. All the routine biochemical labs – the blood counts, the chemistries – are all normal, and it is very frustrating for the oral pathologists, the oral surgeons, and the dentists who manage these patients, because they don't know what to tell the patients. They can't find anything wrong, and yet these patients keep coming in over and over again reporting constant severe mouth pain. Well, now we know one additional (and treatable) entity – MCAS – that can cause this.

Dr. Monthropey: Just a question…we're taught that NSAIDs degranulate mast cells, making things worse. How can they be effective therapy?

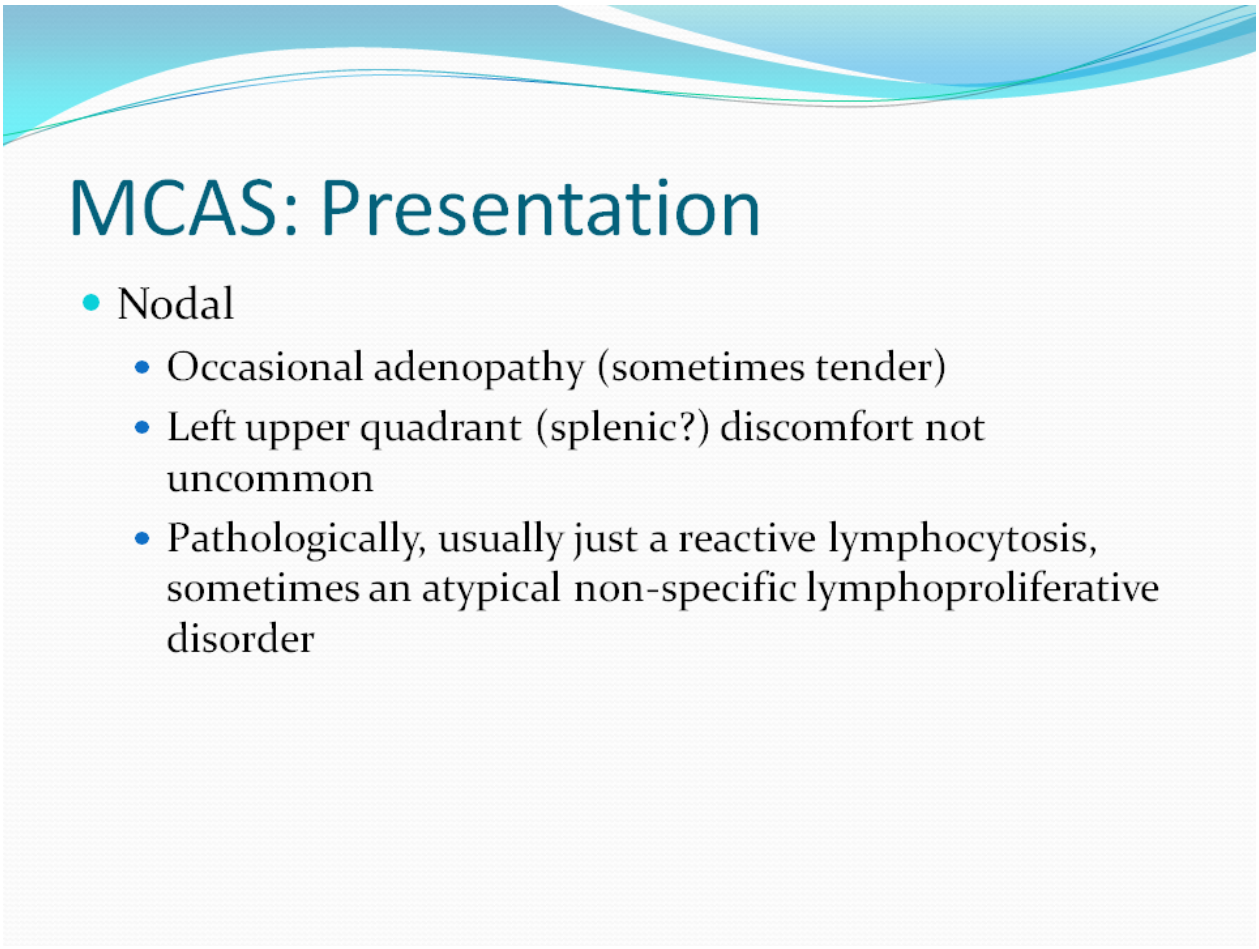
Dr. Afrin: They certainly can make things worse for some patients, and then clearly for other patients they can be inhibitory, though the doses required typically are beyond what most physicians are used to using.

Dr. Sibbald: NSAIDs can be dangerous in mast cell disease. Before we had some of the better long-acting non-sedating H_1 antihistamines, we used to exercise people to deplete their histamine, and often they would clinically improve just through exercising 2-4 times a day.

Dr. Afrin: Of note, the other burning mouth syndrome patients I've found had elevations in mast cell mediators other than chromogranin A and have responded to different

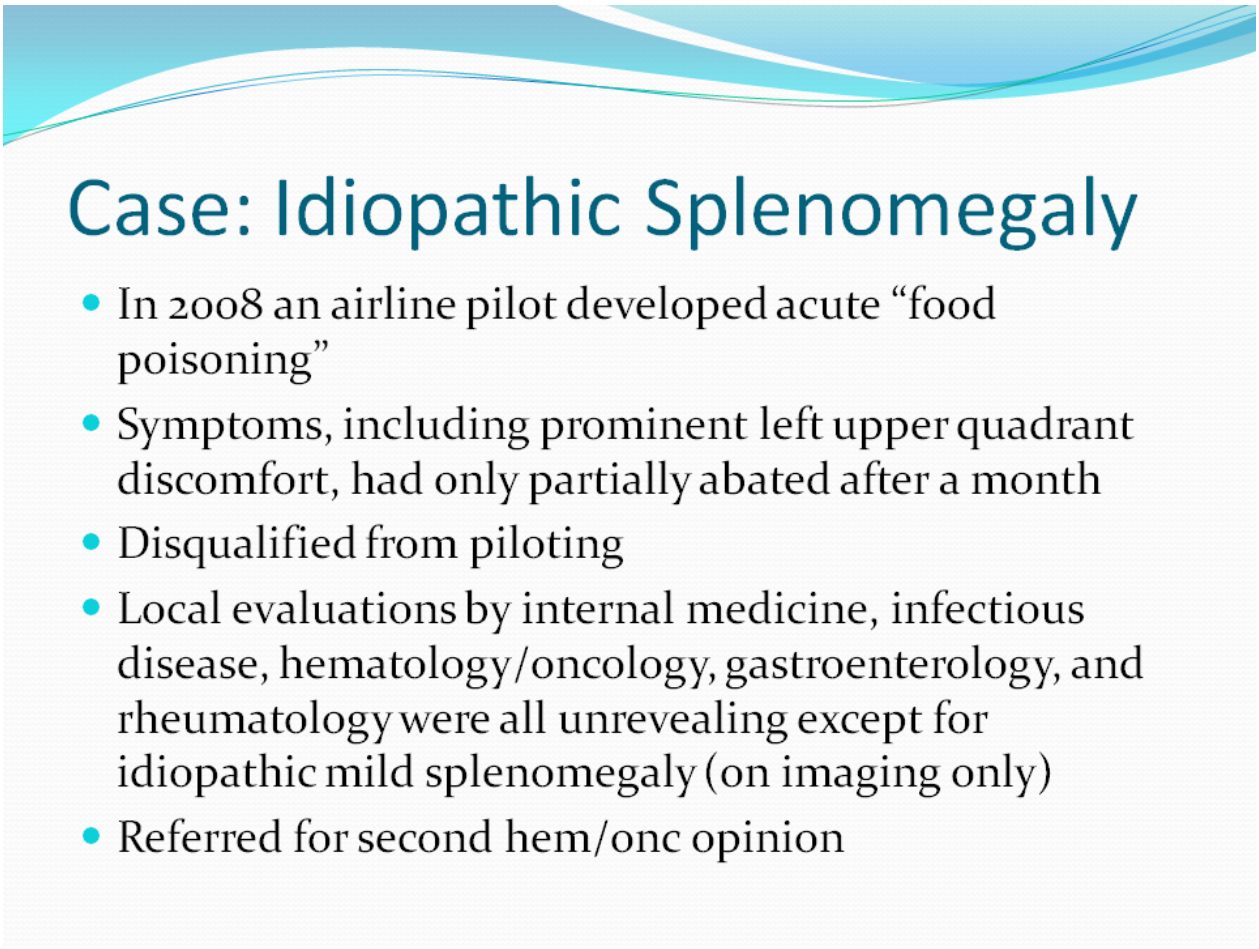
therapies than what worked in my first such patient. I'm not saying that chromogranin A causes the burning sensation or that antihistamines and NSAIDs are the answer to burning mouth syndrome. Different patients have different mutations leading to different mediator expression patterns leading to different assortments of symptoms, and therefore they're going to require different therapies. Clearly, different mediators can lead ultimately to different symptoms, so just because two MCAS patients share a symptom, that doesn't begin to tell you which mediator or combination of mediators is causing it in either patient. My first burning mouth syndrome patient had sky-high chromogranin A levels, but the next five had normal such levels, so it's clearly not the chromogranin A that's causing the symptom. Chromogranin A was just a marker for what was going on in my first burning mouth syndrome patient. I still don't know what the mediators are which were causing her pain.

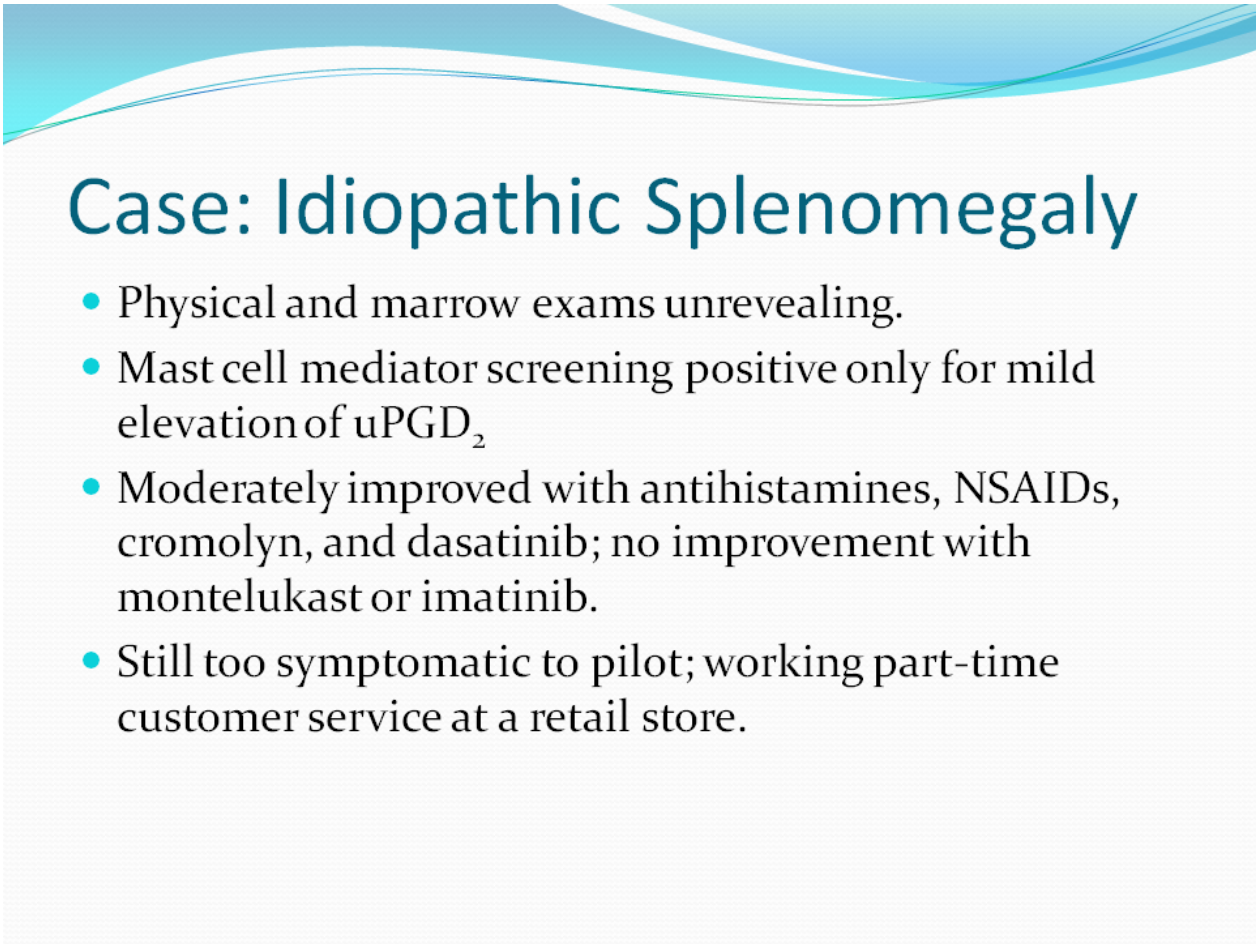
[Slide 51: MCAS: Presentation.]



The nodal issues tend not to be bad. There's occasional adenopathy. Sometimes it's tender, and sometimes there is modest waxing and waning discomfort, particularly in the left upper abdominal quadrant. If you go to the trouble of obtaining biopsies from enlarged and/or tender nodes in MCAS patients, it usually will show only a reactive lymphocytosis. Sometimes the pathologist will go as far as calling it an atypical lymphoproliferative disorder, but he won't commit to calling it a lymphoma, and even though cytogenetic studies may sometimes identify clear clonal abnormalities consistent with lymphoma, it usually won't behave as a lymphoma would be expected behave

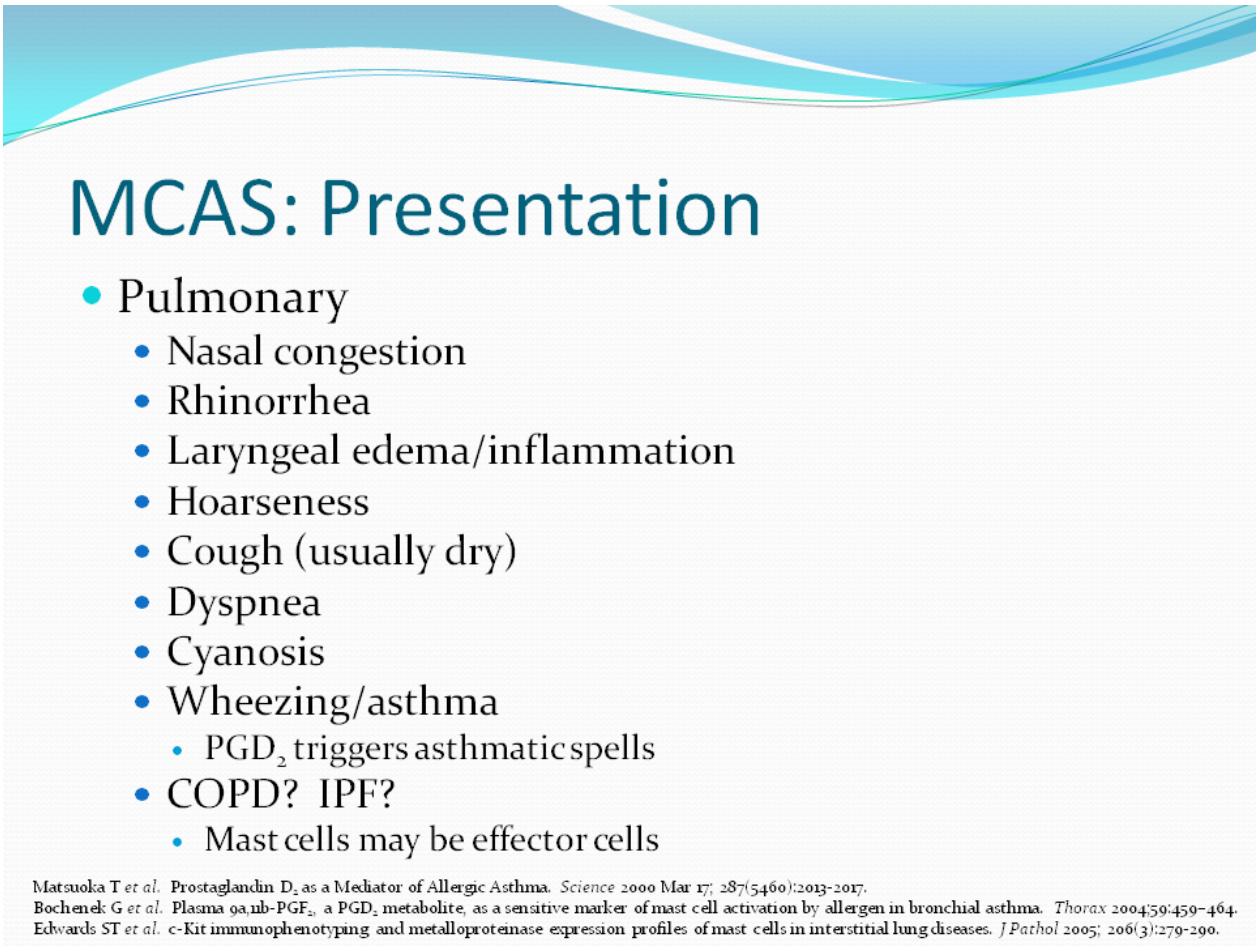
[Slide 52-53: Idiopathic splenomegaly.]





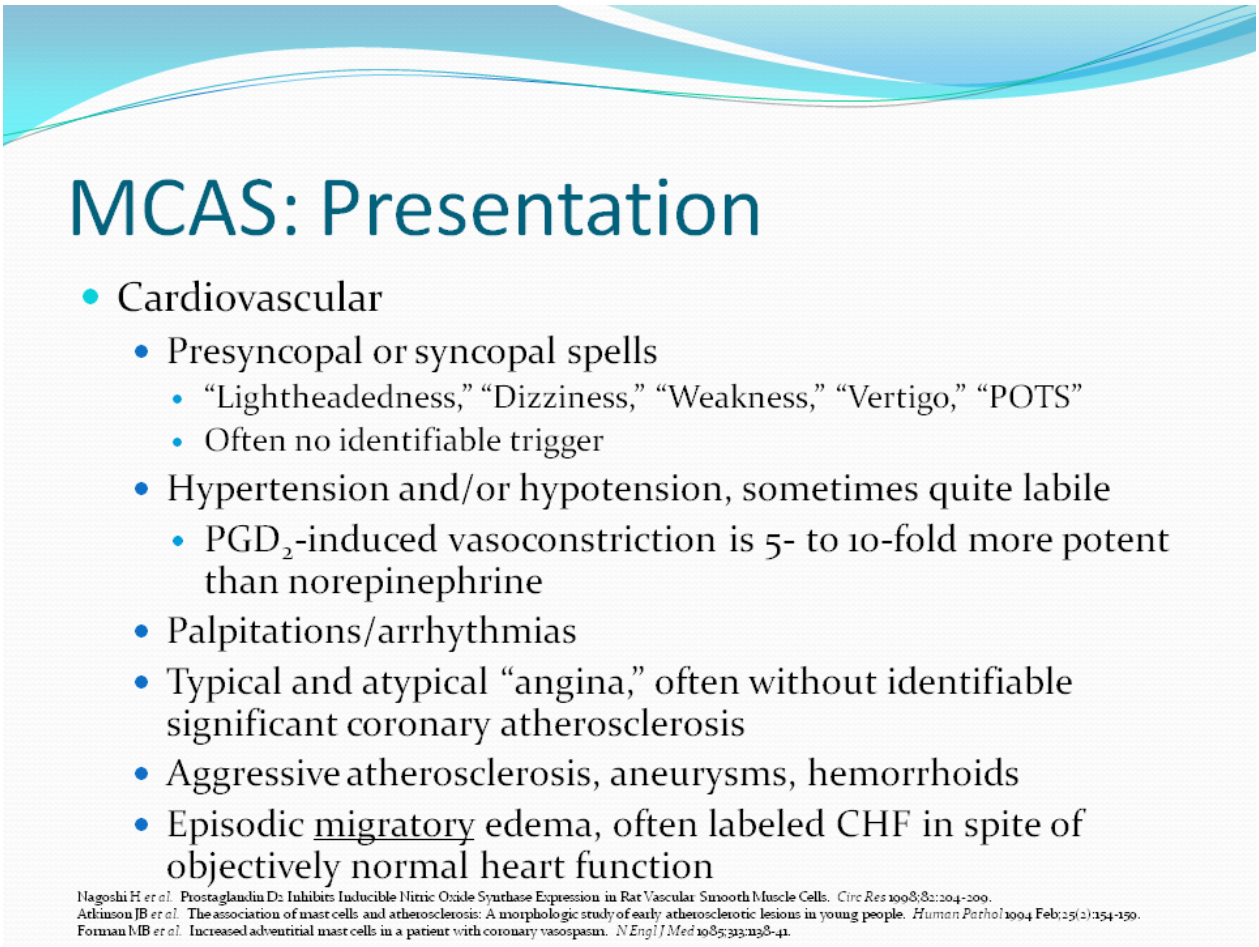
In 2005, an airline pilot in his early 50's developed "acute food poisoning." A lot of MCAS patients will present with that. Not that they haven't had symptoms attributable to MCAS going on for decades, but "food poisoning" sometimes will be their acute presentation. And as will happen with this disease, the food poisoning, if that's what it was, doesn't go away. True food poisoning virtually always goes away, and pretty quickly at that. After a month, his symptoms only partially abated and he was disqualified from piloting, with multiple evaluations all unrevealing except for a tiny bit of enlargement of the spleen for which he got referred to me. Physical and marrow exams were unrevealing, he had a mild elevation of urinary PGD_2, and he has moderately improved with several drugs as you see (in the slides). He's doing a good bit better now, but he's clearly still too symptomatic to be put back in charge of a commercial airliner. I don't want to give anyone the impression that all of my patients are unqualified treatment successes, because they're not. This patient is now working part-time in customer service at a retail store. That's unfortunate, but it's the reality.

[Slide 54: MCAS: Presentation]



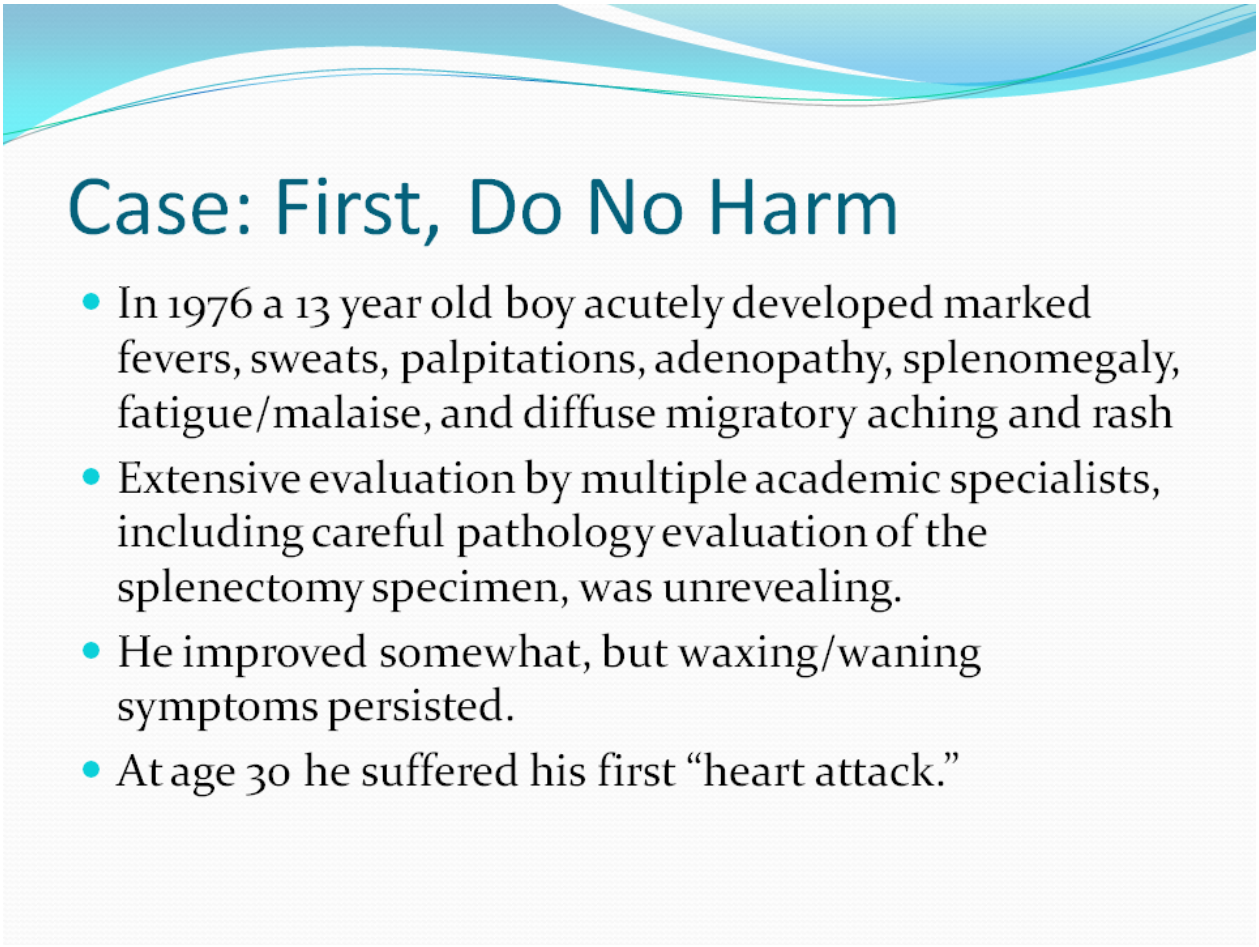
There's a broad range of pulmonary issues that can be seen in MCAS, but what these patients will most commonly complain about in the pulmonary area is something they struggle to find the words to describe. You have to give them the time to figure out how to tell you this, but what they will say in the end is "I just can't get a really deep breath any longer". Extensive pulmonary function testing will be normal, and they'll still tell you, "There is something wrong with my breathing".

[Slide 55: MCAS: Presentation]



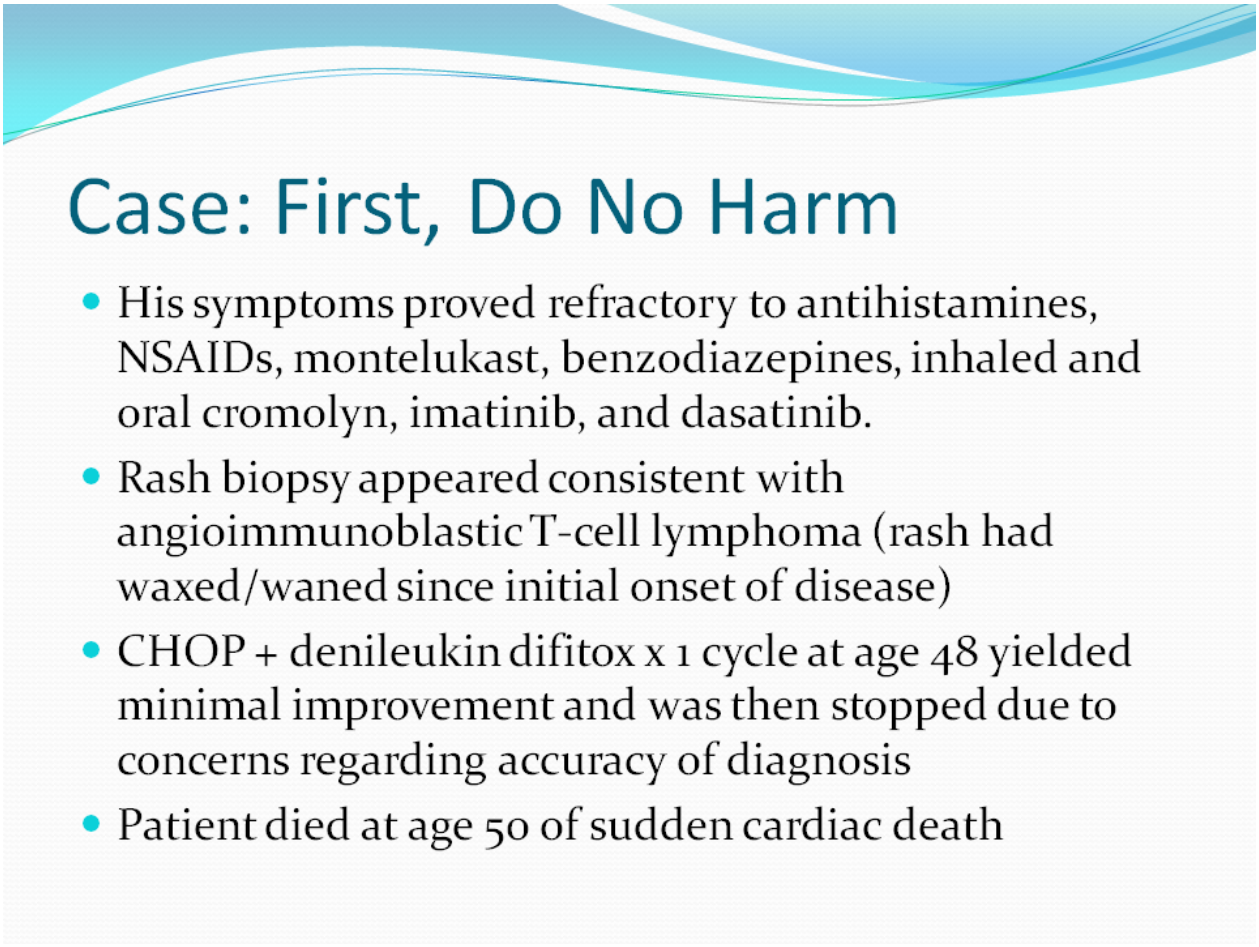
There's a broad range of cardiovascular issues, too. Pre-syncopal and syncopal spells are common, but few patients will use that terminology. They'll use lots of different words – "lightheadedness," "dizziness," "weakness," or "vertigo," and POTS (postural orthostatic tacychardia syndrome) is a very common item on their long problem lists – but if you take a careful history, you will find that some of the POTS spells are orthostatic but many are very clearly not orthostatic. There's often no identifiable trigger. MCAS patients often have both hypertension and hypotension, sometimes quite labile. Palpitations are common. They can have typical and atypical bouts of chest pain that can send them to the emergency room quite frequently, but if they get to the point of being catheterized, you typically find nothing. On the other hand, some of these folks can manifest extremely aggressive atherosclerosis as well as other vascular anomalies that have clearly been linked to mast cell disease, and they will often tell you about this strange pattern of edema – swelling – that's a migratory pattern. It doesn't behave the way heart failure edema behaves and yet these patients, because they complained of swelling, they often get labeled as having heart failure even though they have objectively normal heart function. But heart failure edema doesn't show up as migratory edema.

[Slides 56-58: Case: First, Do No Harm.]



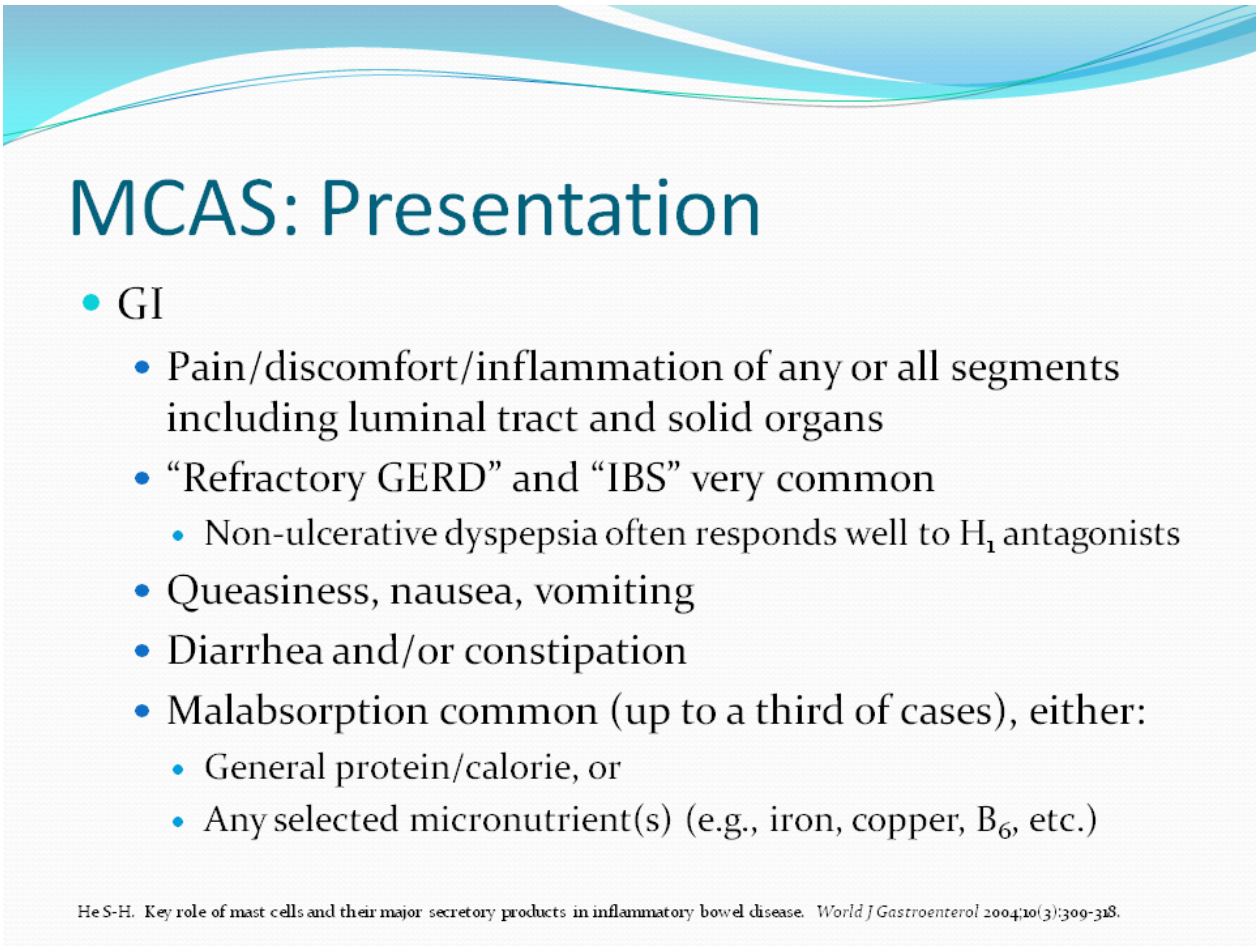
Case: First, Do No Harm

- By age 48 he had suffered >20 "heart attacks" and "strokes," though no evidence of coronary or carotid occlusion was ever found except within the stents that were eventually placed by frustrated cardiologists
- In 2008 he was referred for hem/onc opinion for possible hypercoagulability.
- Extensive evaluation for a defined hypercoaguable syndrome was negative.
- Screening for mast cell disease was negative except for significantly increased mast cells on duodenal biopsy.



In 1976 a 13 year old boy acutely developed marked fever, sweats, palpitations, adenopathy, an enlarged spleen, disabling malaise, diffuse migratory aching, and rash. Extensive evaluation couldn't find any cause. No diagnosis emerged from pathologic analysis of the spleen when it was removed. He continued to have waxing and waning symptoms. At age 30 he suffered his first heart attack, even though catheterization was clean. By 18 years later he had suffered more than 20 "heart attacks" and "strokes," though no evidence of coronary or carotid artery occlusions was ever found except within the coronary stents that his cardiologist had eventually placed out of frustration. Only After stent placement began did he start having definite obstructions, and all of them were in the stents. He was referred to me in 2008. I looked hard for any known hypercoagulable syndrome and could not find one. Screening for mast cell disease was negative except on duodenal biopsy – clearly substantially increased mast cells. Unfortunately he proved refractory to treatment. I'll skip over the details of this. I really was never able to help this patient, and last year at age 50 he died of sudden heart death, probably as a result of occlusion in one of the stents that had been placed by his well-meaning and understandably frustrated cardiologist.

[Slide 59: MCAS: Presentation]



The GI issues again follow on the inflammatory theme in MCAS – pain, discomfort, and inflammation of any or all segments including the luminal tract and solid organs. Refractory GERD and irritable bowel syndrome or inflammatory bowel syndrome are very common. Let me ask you something about refractory GERD patients. These patients are on maximal acid suppression, and yet they continue to complain of a gastritic type of discomfort and reflux. Where is the discomfort coming from if they have no significant acid any longer? My thought is that it never was acid that was causing their problems. They're feeling inflammation. Inflammation hurts regardless of its cause, and though excess acid can be such a cause, in the case of these patients, the inflammation is coming from the release of inflammatory mediators by the mast cell disease. These patients have always had biopsies done, and if you go back to those biopsies and stain them appropriately, you'll likely find histologic evidence of the disease.

Chronic diarrhea (often alternating with constipation) is a common complaint in MCAS and often frustrates their primary doctors, leading them to not uncommonly label the

patients as crazy since, on the surface of it, it would seem there's no way a disease could cause both diarrhea and constipation. Malabsorption, too, is not uncommon, either a general protein-calorie malabsorption that's fairly rare or, much more commonly, there is selective micronutrient absorption deficiency (where the particular micronutrient that's malabsorbed just depends on which mediators are being aberrantly expressed), and if it gets severe enough, the patient then goes on to have the clinical consequences of that micronutrient deficiency which just further complicates the clinical presentation.