CAT
Diagnosis of systemic mastocytosis

Elke Del Biondo, ASO Klinische Biologie
Supervisor: Prof. Dr. N. Boeckx

20-03-2014

UZ Leuven | Herestraat 49 B - 3000 Leuven | www.uzleuven.be tel. +32 16 33 22 11 | UNIVERSITY HOSPITALS LEUVEN

Table of contents

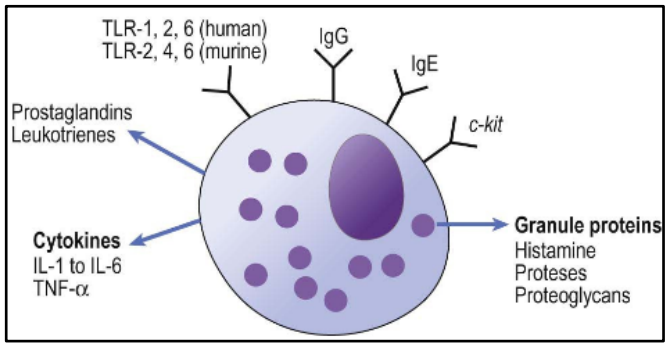
History

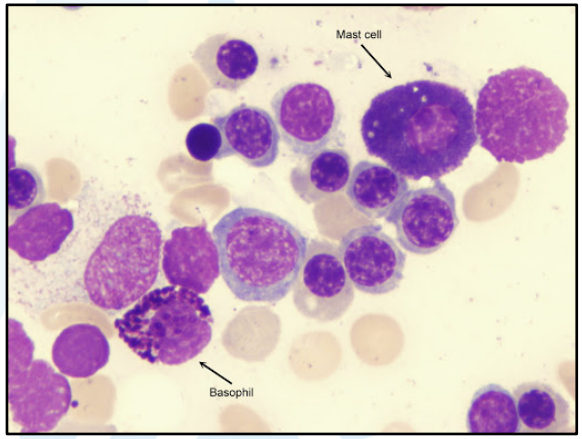
- **1878**: Ehrlich: metachromasia – “mastzellen”
  - Near blood vessels, nerves and glandular ducts
  - 1908: Nobel prize
- **1894**: Unna: urticaria pigmentosa (UP) are associated with increased mast cells below each lesion
- **1931**: Webb: peritoneal mast cells (MC) of rat undergo degranulation following irritation by egg white
- **1930-1940:** Jorpes: mast cells carry heparine
- **1930-1940:** Riley: mast cells carry histamine
- **1949**: Ellis: report of a fatal case of UP in a 1-year-old child. Autopsy showed MC infiltration in skin, liver, spleen, lymph nodes and bone marrow (BM)
- **1950**: MC play a role in anaphylaxis
- Beschrijving verschillende varianten van mastocytose

  → Classificatiesystemen → WHO criteria (laatste update in 2008)

Metcalfe, Blood 2008

Typical features of mast cells





- Granules:
  - Histamine
  - Heparine
  - Tryptase
- Receptors:
  - FcεRI: IgE
  - KIT: stem cell factor
  - TLR
- Cytokines:
  - IL4, IL5
  - TNFα
- Prostaglandins, leukotriënes
- Connective tissue of ≠ organs
  - Skin, gut mucosa, pulmonary alveoli
  - Chorioid plexus, meninges
  - Bone marrow: low numbers

Metcalfe, Blood 2008

Role of mast cells in the human body

- Role in *physiologic state*:
  - Innate defence against viral and bacterial pathogens
  - Acquired immunity (parasitic infections)
  - Effector cells in allergic responses

- Role in *disease*:
  - Allergic disease
  - Mastocytosis
    - Cutaneous mastocytosis (CM)
    - Systemic mastocytosis (SM)

Metcalfe, Blood 2008
UpToDate version 21.1

Epidemiology of systemic mastocytosis

Children	Adults
• Appears in 1st year of life in 80% • Mostly cutaneous forms • Most improve or resolve during adolescence	• Mostly systemic forms • Tend to persist during life

UZ Leuven: ‘zorgprogramma mastocytose’

- Cutaneous mastocytosis: Prof. J. Ceuppens, 19 patients in 2013
- Systemic mastocytosis: Prof. M. Delforge, 13 patients in 2013

Metcalfe, Blood 2008
UpToDate version 21.1

Clinical symptoms of systemic mastocytosis

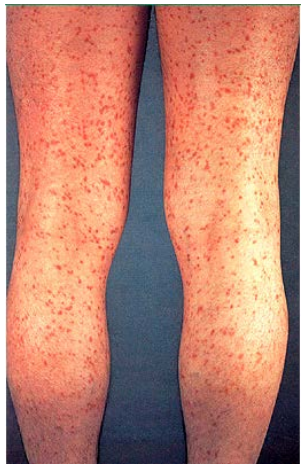
- Cutaneous symptoms
  - Urticaria pigmentosa
  - Dariers sign
  - Diffuse cutaneous mastocytosis
- Symptoms of mediator release
  - Allergic reactions and anaphylaxis
  - Gastrointestinal symptoms

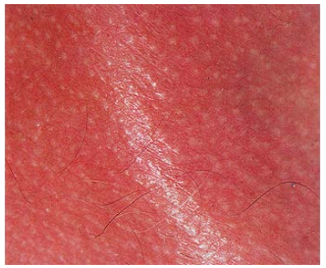
    abdominal pain, nausea, diarrhea, …
  - Neuropsychiatric symptoms

    depression, mood changes, increased somnolence, …
  - Musculoskeletal symptoms

    musculoskeletal pain, osteoporosis
- Symptoms of organ infiltration

  Splenomegaly (+/- hypersplenism), hepatomegaly (+/- ↑ liver enzymes), lymphadenopathy





Metcalfe, Blood 2008
UpToDate version 21.1

Pathogenesis of mastocytosis

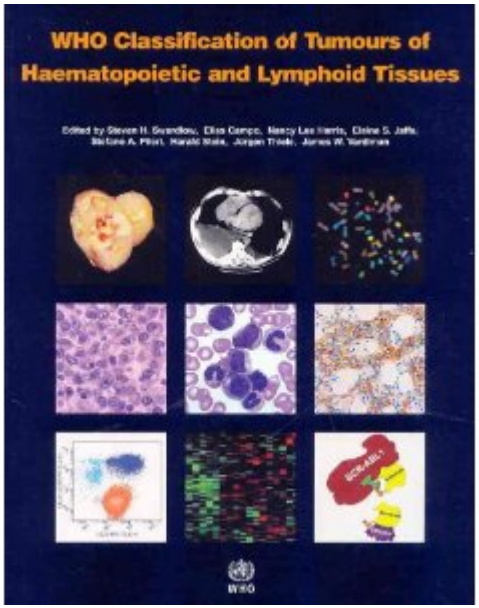
- Activating KIT mutations play a role in CM en SM
- These mutations lead to SCF independent activation of the KIT receptor
- Most common: D816V mutation in exon 17
- > 95% of adults with SM have the D816V mutation in BM cells
- 40% of children with CM have exon 17 mutations, another 40% carry c*KIT* mutations outside exon 17
- Mastocytosis is an acquired disease

Metcalfe, Blood 2008
Fuller, Hematol Oncol Clin N Am 2012

Diagnostic criteria for systemic mastocytosis

WHO 2008 revised criteria

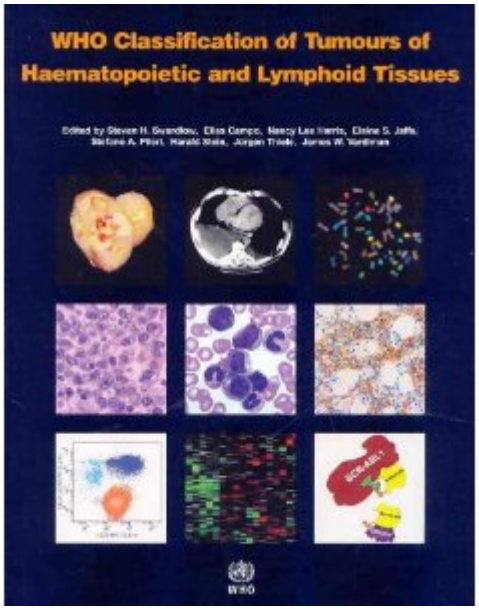
Major criteria
Multifocal, dense infiltrates of MC (>15 MC in aggregates) are detected in sections of BM and/or other extracutaneous organs.
Minor criteria
In biopsy sections of BM or other extracutaneous organs, >25% of the MC in the infiltrate are spindle shaped; have atypical morphologic features; or, of all MCs in BM aspirate smears, >25% are immature or atypical.
Detection of an activating point mutation at codon 816 in *KIT* in BM, blood or another extracutaneous organ.
MC in BM, blood or other extracutaneous organs express CD2 and/or CD25 in addition to normal mast cell markers.
Serum total tryptase persistently exceeds 20 ng/mL (unless there is an associated clonal myeloid disorder, in which case this parameter is not valid).



1 major + 1 minor criterium
or
3 minor criteria

Systemic mastocytosis variants

ISM
Meets the criteria for SM. No C findings and no evidence of an AHNMD. The mast cell burden is low and skin lesions are frequently present. a. Bone marrow mastocytosis: ISM with BM involvement, but no skin lesions b. Smoldering SM: ISM, but with 2 or more B findings, but no C findings
SM-AHNMD
Meets criteria for SM and criteria for an AHNMD (MDS, MPN, MDS/MPN, AML, or other WHO defined myeloid hematologic neoplasm, with or without skin lesions).
ASM
Meets criteria for SM. One or more C findings present. No evidence of mast cell leukemia. Variable involvement by skin lesions.
MCL
Meets criteria for SM. Bone marrow biopsy shows a diffuse infiltration, usually compact, by atypical, immature MCs. Bone marrow aspirate smears show 20% or more MCs. Typical MCL: MC comprise 10% or more of peripheral blood white cells. Aleukemic MCL: <10% of peripheral blood white cells are MCs. Usually without skin lesions.
B findings
Bone marrow biopsy showing >30% infiltration by MCs (focal, dense aggregates) and serum total tryptase level > 200 ng/mL Signs of dysplasia or myeloproliferation in non-mast cell lineage(s), but insufficient criteria for definitive diagnosis of a hematopoietic neoplasm (AHNMD), with normal or only slightly abnormal blood counts Hepatomegaly without impairment of liver function, and/or palpable splenomegaly without hypersplenism, and/or lymphadenopathy on palpation or imaging (> 2 cm)
C findings
Bone marrow dysfunction manifested by 1 or more cytopenia (ANC < 1 x 10^9/L, Hb < 10 g/dL, or platelets < 100 x 10^9/L) Palpable hepatomegaly with impairment of liver function, ascites, and/or portal hypertension Skeletal involvement with large osteolytic lesions and/or pathologic fractures Palpable splenomegaly with hypersplenism Malabsorption with weight loss from gastrointestinal tract mast cell infiltrates



Mast cell activation syndromes

- Patiënten met symptomen van mast cel activatie waarbij geen clonaliteit of allergische basis wordt aangetoond
- Patiënten met bewijs van clonale mastcellen (FCM en/of cKIT mutatie) die niet voldoen aan de criteria voor SM

Mast cell activation syndromes

International Archives of Allergy and Immunology

Definitions, Criteria and Global Classification of Mast Cell Disorders with Special Reference to Mast Cell Activation Syndromes: A Consensus Proposal

Peter Valent[a, b] Cem Akin[c] Michel Arock[d] Knut Brockow[e] Joseph H. Butterfield[f] Melody C. Carter[g] Mariana Castells[c] Luis Escribano[h] Karin Hartmann[i] Philip Lieberman[j] Boguslaw Nedoszytko[k] Alberto Orfao[l] Lawrence B. Schwartz[m] Karl Sotlar[n] Wolfgang R. Sperr[a] Massimo Triggiani[o] Rudolf Valenta[p] Hans-Peter Horny[q] Dean D. Metcalfe[g]

Table 2. Criteria for the diagnosis of MCA

Typical clinical symptoms (see table 3)
Increase in serum total tryptase by at least 20% above baseline plus 2 ng/ml during or within 4 h after a symptomatic period
Response of clinical symptoms to histamine receptor[1] blockers or 'MC-targeting' agents, e.g. cromolyn

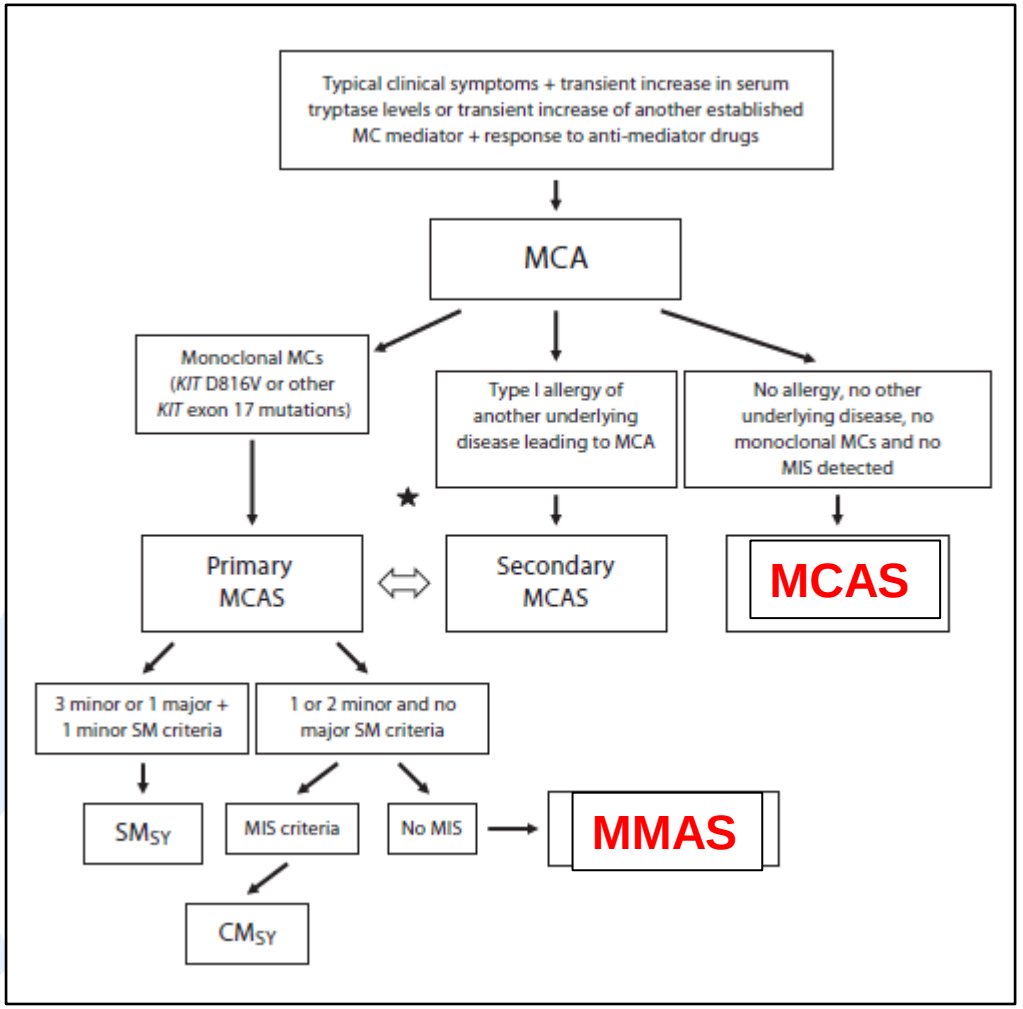
[1] Histamine receptor blockers: HR1 +/– HR2 inverse agonists.

Table 3. Symptoms considered typical for MCA by the members

Symptom(s)	Consensus level
Flushing	95%
Pruritus	90%
Urticaria	85%
Angioedema	75%
Nasal congestion	90%
Nasal pruritus	90%
Wheezing	70%
Throat swelling	85%
Headache	90%
Hypotension	95%
Diarrhea	90%

In order to count as cocriterion of MCA, these symptoms need to be recurrent or permanent, cannot be explained by other known disorders/conditions (other than MCA), and require a therapeutic intervention. Moreover, apart from these symptoms, additional clinical and laboratory criteria have to be fulfilled for the condition/reaction to be considered as MCA.

Mast cell activation syndromes



Valent et al. Int Arch Allergy Immunol 2012

Treatment of systemic mastocytosis

- Avoidance of factors that trigger MC release
  - Sudden temperature changes
  - Aggravated allergic reactions (eg after hymenopthera stings, general anesthesia, …)
- Treatment of symptoms of mediator release
  - Antihistamines
    - H1 blockers: eg cetirizine
    - H2 blockers: eg ranitidine
  - Epinephrine self-injector
- Cytoreductive therapy in case of 1 or more C findings
  - KIT D816V mutants:
    - resistant to Tyrosine Kinase Inhibitors (TKI)
    - interferon-α (IFN-α) + glucocorticoids
  - KIT D816V negative: TKI (eg imatinib)

Questions

1. Which are the diagnostic investigations that should be performed when a diagnosis of SM is suspected?
2. What is the normal and aberrant phenotype for MC?
3. What is the value of flow cytometric detection of CD25 and/or CD2 expression on MC in the diagnosis of SM?

Question 1: Which are the diagnostic investigations that should be performed when a diagnosis of SM is suspected?

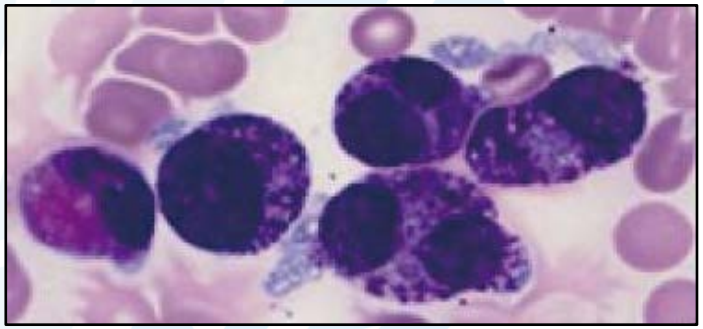
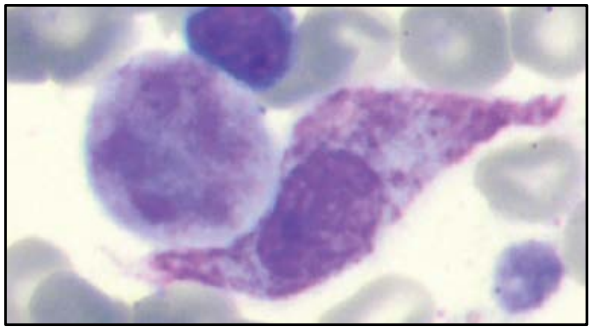
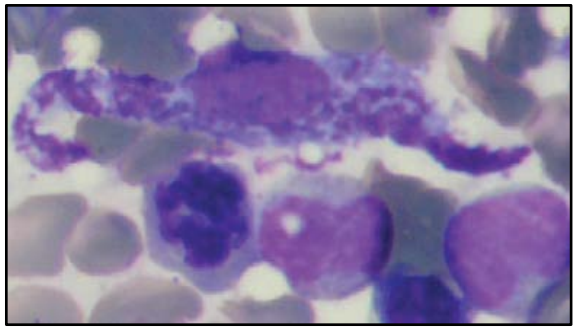
1. Bone marrow histology and immunohistochemistry

- BM trephine biopsy
- Multifocal compact tissue infiltrates of >15 MC = major criterium
- > 25% of MC are immature/atypical= minor criterium
- Immunohistochemical stainings: tryptase, cKIT, CD25
  - MC: tryptase+, cKIT+
  - Aberrant MC: CD25+

Question 1: Which are the diagnostic investigations that should be performed when a diagnosis of SM is suspected?

2. *Bone marrow aspirate cytology*

- >25% of BM MC are immature or atypical = minor criterium
- Number of MC lower than in biopsy (<1%)
- Aberrant MC:
  - spindle shaped, hypogranular, eccentric nuclei
  - bilobar forms

Question 1: Which are the diagnostic investigations that should be performed when a diagnosis of SM is suspected?

3. Abnormal expression of CD2 and CD25 on mast cells

- Immunohistochemistry: - less sensitive
  + easier access
- Flow cytometry: + **highly sensitive** (detects 0,001%)
  - specialized equipment, experience
- CD2 expression = variable, CD25 more reliable
- BM = preferred sample type
- Technical aspects:
  - number of MC very low: > 10^6 events are needed
- PB: in case of MCL, ASM or MC in PB on morphological examination
  - Cave: number of MC in PB usually lower than in BM
  - FACS-based cell sorting needed: not in routine lab

Question 1: Which are the diagnostic investigations that should be performed when a diagnosis of SM is suspected?

4. Serum tryptase levels

- Tryptase level reflects the total burden of MC
- Most SM patients have tryptase > 20 ng/ml
- Limited diagnostic utility in SM-AHNMD

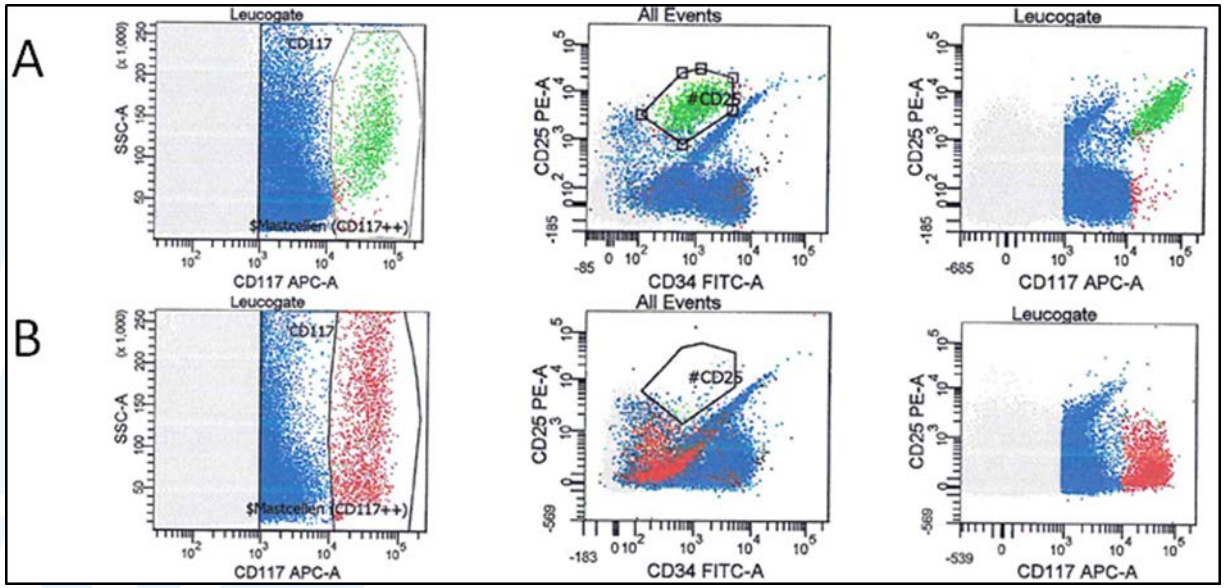
5. Molecular studies (KIT D816V *detection)*

- SM is associated with gain-of-function mutations in KIT tyrosine kinase domain
- 95%: D816V mutations
- Other mutations: V580G, D815K, D816Y, insVI815-816, D816F, D816H and D820G
- Detection by highly sensitive techniques recommended (UZ Leuven: allele-specific PCR: detection limit 0,005%)

Question 2:

What is the normal and aberrant phenotype for MC?

- **Normal** MC are CD33+, CD34-, CD45^{low} and CD117^{high}
- **Aberrant** MC also express CD25, CD2, CD11c, CD35, CD59, CD63 and/or CD69
- Most authors recommend detection of **CD25 and CD2** on MC, however reported sensitivities of CD2 are variable



Gating strategy for mast cells (CD117++, CD34 -) and aberrant mast cells (CD25+). **A**: A distinct population of MC (0,119% of ANC) is present in the **CD117**++ gate. These MC express CD25 which is a minor diagnostic criterion for SM. **B**: A distinct population of MC is present, these MC do not express CD25.

Escribano L et al. Cytometry B Clin Cytomet. 2004

Question 3:

What is the value of flow cytometric detection of CD25 and/or CD2 expression on MC in the diagnosis of SM?

1. Evidence in the literature

Author, year	patients	controls	CD2 and/or CD25 expression	
			sensitivity	specificity
van Daele PLA, 2009	**36** SM	**31** non-SM: - 20 haematological disorders - 4 undiagnosed haematological disorders - 3 bone marrow donors - 4 various diseases (pulmonary hypertension, liver cirrhosis, AIDS)	100%	91%
Pozdnyakova O, 2012	**23** SM - 19 ISM - 2 ASM - 2 SM-AHNMD	**70** non-SM - 1 mast cell sarcoma - 4 CM - 8 MMAS - 18 MCAS - 16 anaphylaxis - 2 angioedema - 2 chronic urticaria - 4 no clinical information	77% (standard) 85% (revised)*	96% (standard) 96% (revised)*
Morgado JMT, 2012	**276** SM - 56 ISM – skin lesions - 196 ISM + skin lesions - 16 ASM - 6 ISM-AHNMD - 2 ASM-AHNMD	**610** non-SM - 519 non-mast cell-related disorders - 51 MMAS - 37 MCAS - 3 mastocytoma	100%	99%

* Revised flow cytometric criteria: a distinct population in the CD117++ gate without CD2 and/orCD25 expression was considered as a positive result for SM.

Question 3:

What is the value of flow cytometric detection of CD25 and/or CD2 expression on MC in the diagnosis of SM?

2. In house evaluation on patient samples

2.1 Materials and methods

- Patients
  - 25 patients with suspected diagnosis of SM
  - Mainly reffered to allergology consultation
  - October 2012 - Februari 2014
  - Exclusion of 2 patients because of incomplete workup (no KIT mutation analysis)
- Bone marrow assesment
  - Trephine biopsies: histopathological investigation
  - BM aspirate smears: cytology
- Immunophenotypic studies
  - FCM according to Escribano et al.
  - Collection of 1 x 10^6 events if possible
  - Gating MC: CD117++ and CD45 dim, Aberrant MC: also CD25 expression
- Molecular studies (*KIT* D816V mutation)
  - Highly sensitive, allele-specific PCR (CME)
- Tryptase
  - Immunocap 250

Question 3:

What is the value of flow cytometric detection of CD25 and/or CD2 expression on MC in the diagnosis of SM?

2. In house evaluation on patient samples

2.2 Results

Patient	Age	Sex	Clinical Symptoms	BB	BM	Tryptase (ng/ml)	p.D816V*KIT* mutation (%)	CD25 (%)	Final diagnosis
1	50	M	anafylaxis	nl	nl	28	0,002	0,020	**ISM**
2	41	F	UP + anafylaxis	SM	nl	20	0,010	0,005	**ISM**
3	53	M	anafylaxis	SM	SM	>200	0,020	0,090	**ISM**
4	40	F	UP	SM	SM	22	0,210	0,033	**ISM**
5	56	M	UP + anafylaxis	SM	SM	29	0,170	0,024	**ISM**
6	6	F	UP + anafylaxis + gastro-intestinal	ND	SM	83,9	pos	0,105	**ISM**
7	71	F	anafylaxis	SM	SM	83	2,17	0,046	**ISM**
8	49	M	UP + anafylaxis	atypical MC	nl	12	0,026	0,008	**ISM**
9	31	F	UP	SM	nl	84,8	0,61	0,021	**ISM**
10	63	M	UP + osteolytic bone lesion	SM	nl	34	0,420	0,038	**ASM**
11	4	M	fever + fatigue + night sweating	nr	SM + AML	107	0,000	1,122	**SM-AHNMD**
12	27	M	anafylaxis	nl	nl	14	0,007	0,020	**MMAS**
13	38	F	urticaria + gastro-intestinal	nl	nl	23	0,000	0,002	**MMAS**
14	52	F	facial erythema + food allergies	nl	nl	6	0,000	0,000	lactose intolerance + rosacea
15	51	F	pruritus + rash	nl	nl	3	0,000	0,000	no final diagnosis
16	50	M	anafylaxis	nl	nl	40	0,000	0,000	**MCAS**
17	46	M	anafylaxis	nl	nl	5	0,000	0,000	no final diagnosis
18	57	F	elevated tryptase + non-specific symptoms	nl	nl	16	0,000	0,000	*H. pylori* gastric infection
19	28	M	gastro-intestinal	nl	nl	22	0,000	0,000	intestinal worm (oxyures)
20	65	F	allergies	nl	nl	14	0,000	0,000	no final diagnosis
21	55	M	urticaria + angio-edema	nl	nl	22	0,000	0,000	**MCAS**
22	56	F	anafylaxis	nl	nl	18	0,000	0,000	no final diagnosis
23	45	F	anafylaxis	nl	nl	15	0,000	0,000	no final diagnosis

Question 3:

What is the value of flow cytometric detection of CD25 and/or CD2 expression on MC in the diagnosis of SM?

2. In house evaluation on patient samples

2.3 Discussion

11 patients meet diagnostic criteria for SM

- 9 ISM
- 1 ASM
- 1 SM-AHNMD

11/11 patients with SM showed expression of CD25 on MC ⇒ **100% sensitivity**

12 patients do not meet the diagnostic criteria for SM

- 2 MMAS
- 2 MCAS
- 1 lactose intolerance
- 1 *H. pylori* gastric infection
- 1 intestinal worm (oxyures)
- 5 patients without a final diagnosis

2/12 patients without SM showed expression of CD25 on MC ⇒ **83% specificity**

= 2 patients with MMAS !

Question 3:

What is the value of flow cytometric detection of CD25 and/or CD2 expression on MC in the diagnosis of SM?

2. In house evaluation on patient samples

2.3 Conclusion

- Highly sensitive flow cytometry is a useful tool in the diagnosis of SM
  - 100% sensitivity in our patient population
  - CD25 expression seems to be reliable as a sole criterion
- A negative result with flow cytometry rules out the diagnosis of SM in our patient population
- ‘false positive results’ in patients with symptoms of MC degranulation and 1 or 2 minor WHO criteria: think MMAS

To Do

- To implement the flow cytometric immunophenotyping of MC on BM (and PB if indicated) as a routine diagnostic tool for the diagnosis of systemic mastocytosis

- To test a control group of patients with various hematological and non-hematological disorders for the presence of CD25 positive mast cells, and correlate the results with the presence of the *KIT* D816V mutation.

Questions…