Critically Appraised Topic

Diagnosis of Systemic Mastocytosis

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Clinical Bottom Line

The diagnosis of systemic mastocytosis (SM) is based on the World Health Organisation (WHO) criteria including one major criterion, namely the presence of multifocal, dense infiltrates of ≥15 mast cells (MC) in bone marrow (BM) and/or extracutaneous organs and four minor criteria: 1) >25% of the MC in a biopsy or BM smear are spindle shaped, immature or atypical; 2) serum tryptase > 20 ng/ml; 3) *KIT* D816V mutation; and 4) expression of CD2 and/or CD25 on MC. One major and one minor criterion or three minor criteria are diagnostic for SM. (1)

For many years, morphologic evaluation has been considered the “gold standard” in diagnosing SM, and the presence of multifocal aggregates of more than 15 MC has become the only major criterion for diagnosing systemic disease. However, in some instances, mast cell aggregates are absent or small. In these cases the diagnosis of SM relies on the presence of 3 or more minor criteria, including the aberrant expression of CD2 and/or CD25 on MC. These antigens can be detected by immunohistochemical methods as well as by flow cytometry. The advantage of flow cytometry is that it is a rapid and sensitive technique. This high sensitivity is achieved by using a multiparameter approach that allows the analysis of aberrant marker expression on specifically gated mast cells.

The flow cytometric analysis of MC was also evaluated on our own patient samples. The results of flow cytometry were correlated with the results of the bone marrow biopsy, cytology of bone marrow smears, serum tryptase levels and molecular analysis (*KIT* D816V mutation).

Clinical/Diagnostic scenario

Mast cells reside in the connective tissue of a variety of tissues and all vascularized organs. They are positioned as sentinels at sites where pathogens might invade the body: the dermis, gut mucosa and submucosa, conjunctiva, pulmonary alveoli and airways. Dermal mast cells are often located in close proximity to blood vessels, nerves and lymphatics. From this strategic location, they can monitor the blood for inflammatory and infectious changes, capture circulating IgE molecules on their surface and distribute their mediators throughout the body. Low numbers of MC are found in the normal bone marrow. (2, 3)

Mast cells develop from pluripotential CD34+ stem cells originating in the bone marrow and spleen. Stem cell factor (SCF), produced by stromal cells, is critical for almost every stage of MC development and survival. The surface receptor for SCF is Kit (CD117). This receptor is expressed by many hematopoietic stem cells early in development, but is lost during maturation by all cell types except mast cells.

MC can be recognized by their numerous cytoplasmatic granules. These granules can be stained by toluidine blue (=metachromatic staining). Another way to identify mast cells is staining with monoclonal antibodies directed against the membrane receptors (Kit and the high-affinity IgE receptor) and the granule proteases (such as tryptase and chymase). The tryptase stain will also stain basophils, but these cells are not normally located in tissues, they contain much less tryptase and the nuclear morphology of basophils and mast cells is different. (2)

Mast cell granules contain many different mediators, including histamine, tryptases, chymases, heparin, etc. The major human mast cell-derived mediators are listed in Figure 1. (3)

Figure 1: Major human mast cell-derived mediators. (Copied from Metcalfe)

Class	Mediators	Physiological effects
Preformed mediators	Histamine, serotonin, heparin, neutral proteases (tryptase and chymase, carboxypeptidase, cathepsin G), major basic protein, acid hydrolases, peroxidase, phospholipases	Vasodilation, vasoconstriction, angiogenesis, mitogenesis, pain, protein processing/degradation, lipid/proteoglycan hydrolysis, arachidonic acid generation, tissue damage and repair, inflammation
Lipid mediators	LTB4, LTC4, PGE2, PGD2, PAF	Leukocyte chemotaxis, vasoconstriction, bronchoconstriction, platelet activation, vasodilation
Cytokines	TNF-α, TGF-β, IFN-α, IFN-β, IL-1α, IL-1β, IL-5, IL-6, IL-13, IL-16, IL-18	Inflammation, leukocyte migration/proliferation
Chemokines	IL-8 (CXCL8), I-309 (CCL1), MCP-1 (CCL2), MIP-1αS (CCL3), MIP1β (CCL4), MCP-3 (CCL7), RANTES (CCL5), eotaxin (CCL11), MCAF (MCP-1)	Chemoattraction and tissue infiltration of leukocytes
Growth factors	SCF, M-CSF, GM-CSF, bFGF, VEGF, NGF, PDGF	Growth of various cell types, vasodilation, neovascularization, angiogenesis

The mediators in the table are examples only. In addition, many mediators are identified in human mast cell lines or primary cultures of human mast cells and may not be produced in vivo.

MC play a role in innate defense against certain bacterial and viral pathogens. Furthermore, they are the effector cells in allergic responses including anaphylaxis, urticaria, rhinitis and atopic dermatitis. Mast cell mediators trigger increased intestinal motility, bronchoconstriction and epithelial sloughing; responses which may have protective effects. However, in excess, these responses are manifestations of allergic disease. (2)

Mastocytosis is due to a clonal, neoplastic proliferation of mast cells that accumulate in one or more organ systems. The clinical presentation of mastocytosis is heterogeneous, ranging from skin-limited

disease **(*cutaneous mastocytosis, CM*)** particularly in pediatric cases where the majority experience spontaneous regression of skin lesions, to a more aggressive variant with extra-cutanueous involvement **(*systemic mastocytosis, SM*)** that may be associated with multiorgan disfunction and shorter survival and is generally seen in adults.

The diagnosis of systemic mastocytosis is based on identification of neoplastic MC by morphological, immunophenotypic and/or genetic (molecular) investigations using well established criteria which are listed in the WHO 2008 document. (Table 1) Systemic mastocytosis can be further classified into ***indolent systemic mastocytosis (ISM)***, ***systemic mastocytosis with an associated hematological non-mast cell disease (SM-AHNMD)***, ***acute systemic mastocytosis (ASM)*** and ***mast cell leukemia (MCL)***. (Table 2) The life expectancy of ISM patients is the same as in the general population and transformation to acute leukemia occurs rarely. The median survival of ASM patients is 2 years. The prognosis of SM-AHNMD depends on the underlying hematological disease; the median survival is 3,5 years. The outlook for MCL is very poor, these patients have a median survival of <6 months. (4,18)

Recently, two new syndromes of mast cell activation have been described: the ***monoclonal mast cell activation syndrome (MMAS)*** and the ***idiopathic mast cell activation syndrome (MCAS)***. MMAS presents with recurrent flushing, gastro-intestinal cramps, hypotension and one or two minor criteria for SM: bone marrow analysis reveals mast cells carrying the *KIT* D816V mutation and/or expressing CD25. In patients with MCAS, no external trigger or mast cell abnormality is found. The diagnosis requires objective evidence for mast cell activation (increased baseline serum tryptase) and exclusion of any other known mast cell activation disorder. (5, 6, 7)

Table 1: World Health Organization diagnostic criteria for systemic mastocytosis (1)

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).

Table 2: Major variants of systemic mastocytosis and B and C findings (1)

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 $< 1 \times 10^9$/L, Hb < 10 g/dL, or platelets $< 100 \times 10^9$/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

The management of all patients with SM includes specific advice regarding the avoidance of factors that trigger MC release and treatment of acute and chronic episodes of MC mediator release. When C findings are present, cytoreductive therapy is necessary to treat organ invasion by MC.

MC can be activated by high and, to a lesser extent, cold temperature. Sudden temperature changes should be avoided by patients. Allergic reactions can be more severe in patients who suffer from both allergy and mastocytosis. Fatal anaphylaxis may follow hymenopthera stings, foods and medications (non-steroidal anti-inflammatory drugs, codeine, narcotics). Consequently, patients with allergies and mastocytosis should be advised to carry an epinephrine self-injector. MC mediator release may be triggered by drugs used in general anesthesia, which is considered a high-risk procedure in patients with mastocytosis. Several peri-operative protocols have been proposed for the management of patients with mastocytosis. These protocols include the avoidance of β-adrenergic blockers, α-adrenergic blockers and cholinergic receptor agonists, and the monitoring of serum tryptase levels and coagulation profiles during surgery and anesthesia. (7)

Cytoreductive therapy is indicated only in the presence of one or more C findings, indicating organ impairment.

The D816V mutant is resistant to KIT kinase inhibition by tyrosine kinase inhibitors (TKI). For patients carrying the KITD816V mutation, interferon-α (IFN-α) in combination with glucocorticoids is used as initial therapy; if no response is seen or if patients are intolerant to IFN-α, treatment is changed to cladirabine. In highly aggressive or relapsed cases, combination chemotherapy followed by a hematopoietic stem cell transplant should be considered. Several chemotherapeutic agents have activity in the treatment of ASM or MCL, including cytarabine, fludarabine and hydroxyurea. (17) Those patients who are negative for D816V but have non-codon 816 mutations or wild-type KIT may show a reponse to TKI, such as imatinib.

In patients with SM-AHNMD, both underlying disease components should be treated as if they existed in isolation. If no C findings can be attributed to SM, no cytoreductive therapy for SM is required. The AHNMD is treated with standard therapy, and complete remissions have been reported in patients with SM and acute myeloid leukemia. (7, 16, 17)

In this CAT, the diagnostic investigations that should be performed when a diagnosis of SM is suspected are reviewed. Secondly, we want to determine the value of flow cytometric detection of CD25 and/or CD2 expression on MC in the diagnosis of SM. We do so by reviewing the literature and by evaluating the flow cytometric technique adapted from Escribano on patient bone marrow samples (since this is the preferred sample type).

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?

Search Terms

"Mastocytosis", "Mast cell activation syndrome", "Mast cells AND immunophenotyping"

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Appraisal

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

At first presentation, a bone marrow biopsy and aspiration should be considered in all adult patients with suspected SM. In children, bone marrow examination should be performed only when signs of SM are present or an AHNMD has been detected in other (non-invasive) tests. (19)

When SM is suspected, the following investigations should be performed:

1. Bone marrow histology and immunohistochemistry

The diagnostic workup for SM usually starts with the examination of a BM trephine biopsy since this site is almost universally involved in adult SM and histological criteria for non-BM extracutaneous involvement in SM have not been widely established yet. Further, BM examination allows the detection of a second hematologic neoplasm, if present. The major diagnostic criterion for SM is the presence of multifocal compact tissue infiltrates of MC. The immunohistochemical diagnosis of SM requires the use of antibodies directed against tryptase, CD117 (KIT) and CD25. Mast cells can be identified as they coexpress CD117 and tryptase. Expression of CD25 indicates a neoplastic phenotype in MC. (19, 20, 21)

2. Bone marrow aspirate cytology

For the cytomorphological examination of BM, the use of both Giemsa-stained and Toluidine Blue-stained bone marrow smears is recommended (20). Cytological aspects may be examined better in the vicinity of BM particles, the actual percentage of MC (given as the percentage of all nucleated cells counted) has to be determined a fair distance from any BM particles. In many patients with SM, the percentage of MC in bone marrow smears is less than 5% or even less than 1%, even if histology records a high infiltration grade. (19) In most cases of SM, bone marrow MC show the following deviations from normal tissue MC: (1) cytoplasmatic extensions (special shapes: spindle or fusiform shapes), (2) oval nuclei with excentric (decentralized) position, and (3) hypogranulated cytoplasm with focal accumulations of granules with or without granule fusions. If two or three of these criteria are fulfilled, the cells are referred to as ‘atypical mast cells characteristically found in SM’. In a smaller group of patients, however, the bone marrow MC resemble mature round (normal) tissue MC with only minor morphological deviations. (20)

3. Abnormal expression of CD2 and CD25 on mast cells

CD25 and/or CD2 expression can be examined by immunohistochemistry and flow cytometry. Immunohistochemistry is reported to be less sensitive than flow cytometry (detects up to 1 in 10^5 events, depending on the number of events analyzed), especially when MC aggregates are not present in the BM biopsy. However, flow cytometry is not available in all centers because it requires specialized equipment and experience. In case of a dry tap, flow cytometry cannot be performed on a BM aspirate. (19) Expression of CD2 on MC has been noted to be variable in SM, and consequently CD25 expression may be a more reliable marker for neoplastic MC. (9)

Generally, the preferred type of sample to be studied is BM since the concentration of MC is usually higher in BM than in peripheral blood (PB) or tissue samples. In case of ASM or MCL, PB can also be used because the number of MC in PB is usually higher in these subtypes. For BM, guidelines on sample collection and preparation techniques and data acquisition have been described. (10) We did not find such guidelines for analysing PB and tissue samples. Some authors describe a technique to detect MC in PB using FACS based cell sorting. (22)

The current medical indications for the immunophenotypic analysis of MC by flow cytometry according to the 'Red Española de Mastocitosis' (REMA) are (12):

1) All adult patients with confirmed or suspected mastocytosis at diagnosis
2) All patients with mastocytosis with suspected involvement by pathologic MC of PB or other tissues
3) Adult patients who have an incidental finding of cutaneous mastocytosis with no clinical symptoms of systemic disease
4) Patients with pediatric-onset mastocytosis when the disease persists after puberty if changes (eg, marked increased serum tryptase levels or organomegaly) suggesting progression into an adult form develop
5) Follow-up of patients with adult-onset mastocytosis when disease progression is suspected because of significant changes in the blood cell counts or white blood cell differential, increase in the size of liver or spleen, presence of circulating PB MC, or a marked increase in serum tryptase levels
6) Follow-up of residual disease in patients undergoing MC cytoreductive therapy

4. Serum tryptase levels

The level of serum tryptase reflects the total burden of MC. In healthy controls, tryptase levels average 5 ng/ml (range: < 1 to 15 ng/ml). In most patients with SM, serum tryptase levels exceed 20 ng/ml. The levels of serum tryptase vary widely but are usually higher in ASM and SM-AHNMD patients, who can exhibit levels > 200 ng/ml. Serum tryptase levels are also elevated in a significant proportion of cases with AML, CML and MDS, consequently this test has limited diagnostic utility in the presence of a second SM-associated myeloid neoplasm. (8)

5. Molecular studies (KIT D816V *detection)*

Mastocytosis is associated with a somatic gain-of-function mutation in the *KIT* tyrosine kinase domain. Particularly the D816V mutation can be found in > 95% of adult cases. Other less common (<5%) somatic KIT mutations identified in SM include V580G, D815K, D816Y, insVI815-816, D816F, D816H and D820G. (4) Outside a research setting, it is currently not standard practice to screen for other mutations than those involving D816V. Sensitivity of *KIT*D816V detection may be enhanced by enriching lesional MC by laser capture micro dissection, magnetic bead or FACS-based cell sorting, or through the use of highly sensitive PCR techniques such as an allele-specific real-time quantitative PCR (detects up to 0,005% mutated cells). (11)

Attempts at validating the WHO diagnostic criteria revealed that 20% of ISM patients lack mast cell clusters in the BM and 30% do not exhibit a serum tryptase above 20 ng/ml. In contrast, the sensitivity for detecting morphological atypia, aberrant CD2 and/or CD25 expression or *KIT* D816V

mutation in BM mast cells exceeds 90% when sensitive assays are used, thereby illustrating the increasing importance of these minor criteria in diagnosing SM.

Question 2: What is the normal and aberrant phenotype for MC?

Normal MC express the CD117 antigen and the FcεRI. Neither of these antigens is lineage specific for MC. FcεRI is also present in human basophils and CD117 expression has been reported in BM hematopoietic precursors including myeloid and erythroid committed progenitor cells. To distinguish CD117+ MC from other CD117+ hematopoietic cells, additional markers are used. MC are CD33+, CD34-, $CD45^{low}$ and $CD117^{high}$. (10, 12)

Among the antigens showing aberrant expression on MC from mastocytosis, CD25 appears to be the most sensitive, specific and easy to use because it is negative in normal MC and positive in mastocytosis. Aberrant expression of at least one additional marker (CD2, CD11c, CD35, CD59, CD63 and/or CD69) should also be confirmed. Most authors recommend the simultaneous detection of CD25 and CD2 expression to identify aberrant MC. (10) However, a recent study of Morgado et al. examined the sensitivity and specificity of CD2 versus CD25 expression in the diagnosis of SM and found that the 'CD25+ and/or CD2+' criterion showed an overall sensitivity of 100% and specificity of 99,0% for the diagnosis of SM whereas CD25 expression alone showed a similar sensitivity (100%) with a slightly higher specificity (99,2%). This study analyzed mast cells on 886 bone marrow and 153 non-bone marrow extracutaneous tissue samples by multiparameter flow cytometry. It was also noteworthy that in extracutaneous tissues other than BM, the sensitivity and specificity observed for CD25 expression (77,8% and 96,8% respectively) and for CD2 (40,7% and 85,7% respectively) were considerably lower than those observed in BM samples. The authors suggest that the 'CD25+ and/or CD2+' WHO minor criterion should be updated to a major criterion based exclusively on CD25 expression. (9)

Question 3: What is the value of flow cytometric detection of CD25 and/or CD2 expression on MC in the diagnosis of SM?

To answer this question, a review of the literature and an in house evaluation of flow cytometric detection of CD25 on patient samples were performed.

1. Evidence in the literature

A PubMed search using the terms "mast cell AND immunophenotyping" yielded 3 studies examining the sensitivity and specificity of mast cell immunophenotyping in bone marrow samples. (9, 13, 14) Sensitivities vary between 77% and 100% and specificities between 91% and 99% for CD2 and/or CD25 detection. We notice that the study of Pozdnyakova et al. showed a notable lower sensitivity than the two other studies (77% vs. 100% respectively). This may be due to technical aspects of the flow cytometric analysis (the specific clones used are not mentioned in the article). We also question the 'revised flow cytometric criteria' used by the authors, because MC without CD2 and/or CD25 expression are not aberrant MC. A general remark regarding these studies is the use of a control population consisting of various haematological disorders. In our opinion, only a bone marrow donor can be a 'true negative' for SM, patients with other haematological diseases might have a masked form of SM-AHNMD.

Table 3: Overview of the studies examining the sensitivity and specificity of MC immunophenotyping

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.

2. In house evaluation of the highly sensitive flow cytometric detection of CD25 on patients with a suspected diagnosis of SM

2.1 Materials and methods

Patients

We included 25 patients with a suspected diagnosis of SM, who were mainly referred to the consultation of allergology at UZ Leuven between October 2012 and Februari 2014 with clinical symptoms possibly indicating SM. The clinical presentation and results of bone marrow, immunophenotypic, molecular and biochemical studies were retrospectively reviewed in the patient files. After reviewing the patient files, two patients were excluded because the diagnostic workup was incomplete: *KIT* D816V mutation analysis was not performed. The diagnosis and classification of SM was made using the WHO criteria.

Bone marrow assessment

Trephine biopsies were performed in all cases but one (a six years old girl meeting four minor criteria for SM). BM aspirate smears were performed in all patients. For both investigations, the standard methods in our lab were used.

Immunophenotypic studies

A protocol for immunophenotypic analysis of MC was developed according to a proposal by Escribano et al. (10) BM was stained with CD34 FITC, CD117 APC, CD45 PerCP and CD25 PE antibodies (all from BD Biosciences, San Jose, USA). Samples were analyzed on a FACSCanto II flow cytometer (BD Biosciences, San Jose, USA) collecting ≥ 1 x 10^6 events. MC were identified by their high expression of CD117, intermediate CD45 expression and variable light-scattering characteristics. Co-expression of CD25 identified aberrant MC. CD2 measurement was not performed because the additional value remains uncertain. (9) Figure 2 shows an example of a positive and a negative flow cytometric result.

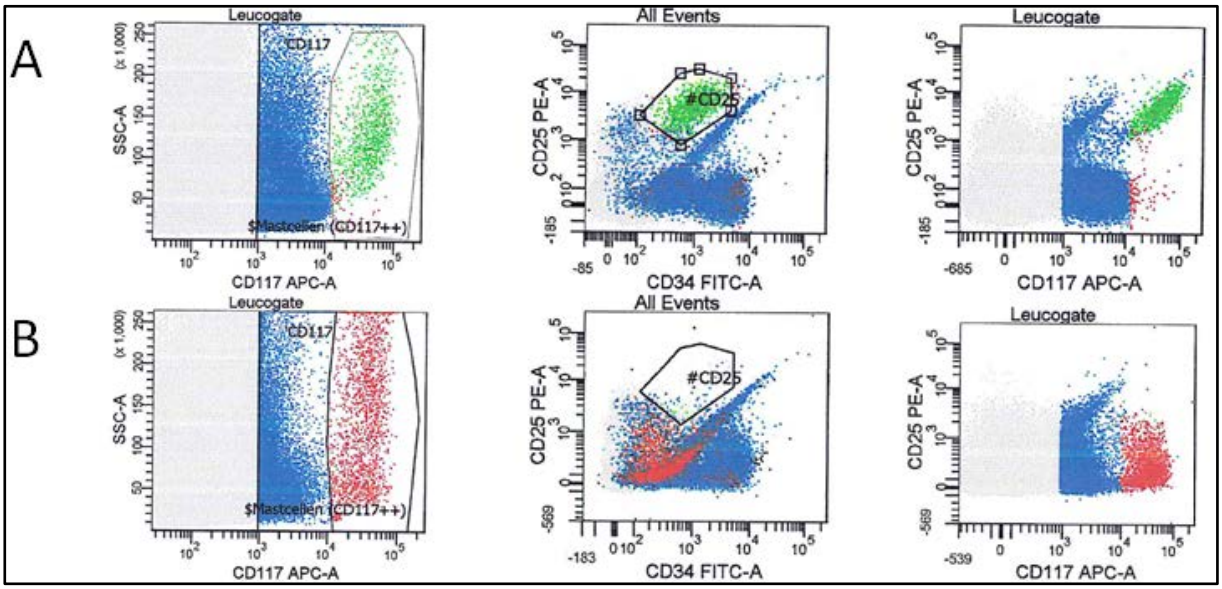
Molecular studies

KIT D816V mutation analysis was performed on all BM aspirates. *KIT* D816V was evaluated using an allele-specific real-time quantitative PCR (qPCR) assay performed on a 7900 Real-Time PCR System from Applied Biosystems. (11) This test was developed and validated at the center for human genetics (CME). The detection limit of this test is approximately 0,005% mutated cells in a wildtype background.

Tryptase

Serum tryptase levels were measured using the Immunocap 250 system (Phadia).

Figure 2: 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 (0,105% of ANC) which is a minor diagnostic criterion for SM. **B**: A distinct population of MC is present, these MC do not express CD25.



2.2 Results

The results of our new flow cytometric method on 23 patient samples and the other diagnostic investigations for SM are summarized in Table 4.

11 patients meet the diagnostic criteria for SM. These patients can be further classified into 9 ISM, 1 ASM and 1 SM-AHNMD. 12 patients do not meet the criteria for SM, the final diagnosis of these patients include 2 MMAS, 2 MCAS, 1 lactose intolerance, 1 H. pylori gastric infection, 1 intestinal worm (oxyures) and 5 patients without a final diagnosis.

13 patients show CD25 expression on the MC. The percentage of CD25 expression on the MC ranges between 0,002% and 1,122% (mean 0,118%). The percentage of MC in the BM aspirates of the CD25+ patients range between 0,011% and 1,207% (mean 0,146%). 11 of these 13 patients meet the diagnostic criteria for SM (sensitivity 100%, specificity 83%). Two patients with CD25 positive MC showed clinical symptoms of MC degranulation but met only 2 WHO criteria for SM: one had also the p.D816V mutation, the other had tryptase >20 ng/ml. Both cases might be considered as having monoclonal mast cell activation syndrome (MMAS).

Table 4: Results of the diagnostic workup of 23 patients with a suspected diagnosis of SM. The grey shaded areas fulfill a WHO criterion.

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

UP: urticarial pigmentosa
nl: normal
ND: the analysis was not performed
nr: the result was not representative

2.3 Conclusion

Highly sensitive flow cytometric immunophenotyping is a useful tool for diagnosing SM by assessing a minor diagnostic WHO criterion with high sensitivity and specificity. The literature reports a high sensitivity (100% by most authors) for the flow cytometric detection of CD2 and/or CD25 on MC. We confirmed these results in our patient population, although we only determined CD25 expression. The negative predictive value of flow cytometry was 100% in our patient group, indicating that a negative flow cytometric result probably rules out the diagnosis of SM.

If <3 minor criteria are fulfilled and patients show clinical signs of MC degranulation, CD25 expression on MC indicates the presence of MMAS.

To Do/Actions

- 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.
- 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