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# Immunophenotyping of mast cells: a sensitive and specific diagnostic tool for systemic mastocytosis

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## ABSTRACT

**Introduction: The diagnosis of systemic mastocytosis (SM) is based on a combination of major and minor criteria. Flow cytometric detection of aberrant expression of CD2 and/or CD25 on CD117-positive mast cells is one of the minor criteria used. In the present study we examined the sensitivity and specificity of mast cell immunophenotyping in the diagnosis of SM.**

**Material and methods: Patients were 36 persons with systemic mastocytosis diagnosed according to WHO criteria. Controls were 31 patients without SM. Immunophenotyping was performed according to published guidelines.**

**Results: All patients with SM were positive for CD2 and/or CD25. All patients without SM, except one, were negative for these markers. The sensitivity for immunophenotyping was 100%, the specificity 91%. The positive and negative predictive values were 97% and 100% respectively.**

**Conclusion: Immunophenotyping of bone marrow derived mast cells is not only a very sensitive but also a very specific method to diagnose SM with high positive and negative predictive value.**

## KEYWORDS

Immunophenotyping, mastocytosis, sensitivity, specificity, tryptase

## INTRODUCTION

Systemic mastocytosis (SM) is a disease characterised by an accumulation of mast cells in one or more organs with a variable clinical course. Manifestations of the disease are largely provoked by the resultant increase in mast cell-derived mediators, which have a variety of local and systemic effects. Diagnosis is based on a combination of major and minor criteria (*table 1*). If at least one major and one minor or at least three minor SM criteria are fulfilled, the diagnosis of SM is made.[1]

Flow cytometric immunophenotyping offers a rapid, objective, and sensitive multiparameter analysis of high numbers of cells from a sample, with information being provided on a single-cell basis. Recent flow cytometric studies have demonstrated that normal human mast cells exhibit a myeloid immunophenotype characterised by the expression of CD117 and CD33 in the absence of reactivity for CD34, CD14, CD15 and lymphoid-lineage-associated markers.[1-3] Mast cells from mastocytosis patients display unique immunophenotypic characteristics, including lowered expression of CD117, aberrant expression of CD2 and/or CD25, and increased expression of CD11c, CD35, CD59, CD63 and CD69.[1-3] Particularly the aberrant expression of CD2 and/or CD25 is of great relevance in the diagnosis and differential diagnosis of SM and consequently flow cytometric detection of CD2 and/or

**Table 1.** *Diagnostic criteria for systemic mastocytosis[1]*

**Major criteria**
- Multifocal dense infiltrates of MC detected in sections of bone marrow and/or of other extracutaneous organ(s)

**Minor criteria**
- In MC infiltrates detected in sections of bone marrow or other extracutaneous organs, >25% of MC are spindle shaped or: in bone-marrow smears, atypical MC comprise >25% of all MC
- Detection of a *c-KIT* point mutation at codon 816 in bone marrow or blood or other extracutaneous organs
- Kit+ MC in bone marrow or blood or other extracutaneous organ(s) coexpress CD2 and/or CD25
- Serum total tryptase concentration persistently >20 µg/l

**If one major and one minor or three minor criteria are fulfilled, then the diagnosis is systemic mastocytosis**

CD25 on CD117 positive mast cells is now one of the minor criteria used to diagnose SM.[1]
In this study, we examined the sensitivity, specificity and positive and negative predictive value of immunophenotyping for the diagnosis of SM.

## MATERIAL AND METHODS

### Patients

The study was conducted at the Department of Internal Medicine and the Department of Immunology of Erasmus University Medical Centre, the Netherlands. Included in the present study are all 36 consecutive patients seen between January 2003 and May 2007 for whom immunophenotyping was performed as part of the diagnostic work-up for SM in our centre. Immunophenotyping is performed routinely since January 2003 in all patients presenting with a suspicion of SM and for whom a bone marrow examination is regarded necessary. Characteristics of the patients are listed in *table 2*. Based on the presence or absence of so-called B- and C-findings, and the presence or absence of other haematological non-mast cell disease in the bone marrow, the patients were subdivided.[1] The majority of patients (n=31) suffered from indolent SM (no B- or C-findings present). Two

**Table 2.** *Characteristics of patients with systemic mastocytosi*

| Age | Sex | Diagnosis | Biopsy | Smear | c-KIT D816V | Tryptase | CD2 | CD25 | % mast cells in BM |
|---|---|---|---|---|---|---|---|---|---|
| 58 | F | ISM | + | + | + | 141 | + | + | 0.1 |
| 57 | F | ISM | + | + | + | 25.5 | + | + | 0.03 |
| 45 | F | ISM | + | + | + | 182 | + | + | 0.04 |
| 50 | F | ISM | + | + | + | 221 | - | + | 0.3 |
| 42 | F | ISM | + | + | + | 179 | + | + | 0.3 |
| 50 | F | ISM | + | + | + | 27.2 | + | + | 0.1 |
| 62 | F | ISM | + | + | + | 47.4 | + | + | 0.03 |
| 53 | F | SSM | + | + | + | 244 | + | + | 0.8 |
| 60 | F | ISM | + | + | + | 29 | + | + | 0.08 |
| 76 | F | ISM | + | + | + | 292 | + | + | 0.04 |
| 50 | F | ISM | + | + | . | 29.4 | + | + | 0.06 |
| 51 | F | ISM | + | + | - | 32.5 | + | + | 0.02 |
| 64 | F | ISM | + | + | - | 123 | + | + | 0.08 |
| 36 | F | ISM | + | + | - | 33.4 | + | + | 0.05 |
| 76 | F | ISM | + | - | + | 20.7 | + | + | 0.11 |
| 51 | F | ISM | . | + | + | 22.3 | + | + | 0.06 |
| 41 | M | ISM | + | + | + | 26.9 | + | + | 0.2 |
| 65 | M | ISM | + | + | + | 42.9 | + | + | 0.8 |
| 49 | M | ISM | + | + | + | 65.6 | + | + | 0.07 |
| 53 | M | ISM | + | + | + | 113 | + | + | 0.5 |
| 45 | M | ISM | + | + | + | 14.1 | + | + | 0.06 |
| 64 | M | ASM | + | + | + | 36 | + | + | 0.1 |
| 49 | M | AHNMD | + | + | + | 24.4 | + | + | 0.07 |
| 49 | M | SSM | + | + | + | 453 | + | + | 0.6 |
| 35 | M | ISM | + | + | + | 81.3 | + | + | 0.13 |
| 45 | M | ISM | + | + | + | 49 | + | + | 1 |
| 50 | M | ISM | + | + | + | 115 | + | + | 0.8 |
| 52 | M | ISM | + | + | . | 106 | + | + | 0.06 |
| 43 | M | ISM | + | + | - | 22.9 | + | + | 0.02 |
| 76 | M | ASM | + | - | + | 212 | + | + | 0.01 |
| 59 | M | ISM | + | - | + | 30.1 | + | + | 0.02 |
| 47 | M | ISM | - | + | + | 28.8 | + | + | 0.05 |
| 61 | M | ISM | - | + | + | 24.8 | + | + | 0.06 |
| 66 | M | ISM | - | + | + | 24.2 | + | + | 0.14 |
| 41 | M | ISM | - | + | + | 14.4 | + | + | 0.1 |
| 62 | M | ISM | - | + | - | 44.9 | + | + | 0.08 |

**M = male; F = female; ISM = indolent systemic mastocytosis; ASM = aggressive systemic mastocytosis; SSM = smouldering systemic mastocytosis; AHNMD = associated haematological non-mast cell disease. + = positive; - = negative; . = missing.**
**For the column 'biopsy' + means: multifocal dense mast cell infiltrate present. For the column 'smear' + means: >25% spindle shaped mast cells present.**

patients had a smouldering SM (B-symptoms present but C-findings absent), two patients presented with aggressive SM (C-findings present) and one patient presented with associated haematological non-mast cell disease.

**Controls**

Controls were 31 patients from the same university hospital without SM, undergoing bone marrow examination for various reasons between March and May 2007. In most cases the control subjects were diagnosed with a haematological disorder (n=20). Four controls were suspected to be suffering from a malignant haematological disorder, but no diagnosis could be made. Three controls had no underlying disease. They underwent bone marrow examination prior to potential bone marrow donation. One of the controls underwent bone marrow examination for reason of an elevated serum tryptase following an allergic reaction. The other controls had various diseases such as pulmonary hypertension, AIDS and liver cirrhosis.

**Immunophenotyping**

Immunophenotyping was performed using flow cytometry according to guidelines published by the Spanish mastocytosis network.[1] Briefly, bone marrow aspirates were collected in heparin tubes and processed within 24 hours. After lysis of erythrocytes using ammonium chloride (pH 7.4) (10 minutes at room temperature (RT)), leucocytes were washed with PBS/BSA and a cell suspension of 60 million/ml was made. Fifty microlitres of this suspension was subsequently stained with antibodies (10 minutes at RT). Antibodies used are: CD117-PE (104D2), CD45-PerCP (2D1), CD25-APC (2A3), CD117-PE-Cy7 (104D2; custom conjugated), CD34-APC-Cy7 (8G12; custom conjugated) (all from BD Biosciences), CD2-FITC (T11), CD2-PE (T11), CD33-PE (My9) (all from Beckman Coulter), and CD117-APC (104D2; Dako Cytomation, Glostrup, Denmark). After incubation, cells were washed with PBS/BSA and resuspended in FACSFlow solution (BD Biosciences). Data were acquired on a FACSCalibur or FACS Canto B; one million cells were acquired per tube. Data were analysed using Paint-a-Gate Pro or FACS Diva software. Mast cells were gated based on strong CD117 expression; in the six-colour analysis additional gating was performed using CD33 positivity and CD34 negativity.

**Other diagnostic tests**

c-KIT mutations (particularly the D816V mutation) were analysed as described previously.[4] Serum tryptase levels were determined using a commercial fluorescent enzyme immunoassay (UniCAP assay and UniCAP 100 instrument; Phadia, Nieuwegein, the Netherlands) according to the manufacturer's instructions. Morphological analysis of bone marrow smears and iliac crest biopsies was performed using standard methods.

## RESULTS

**Immunophenotypic analysis of mast cells**

In all patients with SM, mast cells were detected in the bone marrow aspirate (mean 0.21%; range 0.01 to 1.0%). The number of mast cells in SM patients was significantly higher than in control samples (mean: 0.05%; range 0.00 to 0.72%; p value for difference 0.005).

All patients with SM showed expression of either CD2 or CD25 or both on CD117-positive bone marrow mast cells. CD25 was present on mast cells of all SM patients. CD2 (either conjugated with FITC or PE) was absent on mast cells from one patient with SM. One control patient diagnosed with chronic myelomonocytic leukaemia was positive for CD25, but negative for CD2. Although this patient had a slightly elevated serum tryptase (16.0 µg/l) and an increased number of aberrant mast cells in the bone marrow aspirate, this patient did not fulfil the criteria for a diagnosis of SM, as he had no major and only two minor criteria. D816V mutation analysis was negative in this patient.

Two patients had three positive criteria only after including immunophenotyping as a diagnostic criterion. For the calculation of sensitivity and specificity these two patients were left out of the analysis.

In our patient group sensitivity for CD25 positivity was 100% and specificity was 91%. Negative and positive predictive value were 100 and 97% respectively (*table 3*).

**Table 3.** *Sensitivity, specificity, positive and negative predictive value of immunophenotyping*

| | **Immunophenotyping** | | |
|---|---|---|---|
| | *CD25+* | *CD25-* | *Total* |
| SM | 34 | 0 | 34 |
| Not SM | 1 | 30 | 31 |
| Total | 37 | 30 | 65 |
| *Sensitivity* | *Specificity* | *Positive predictive value* | *Negative predictive value* |
| 100% | 91% | 97% | 100% |

**Other diagnostic criteria for SM**

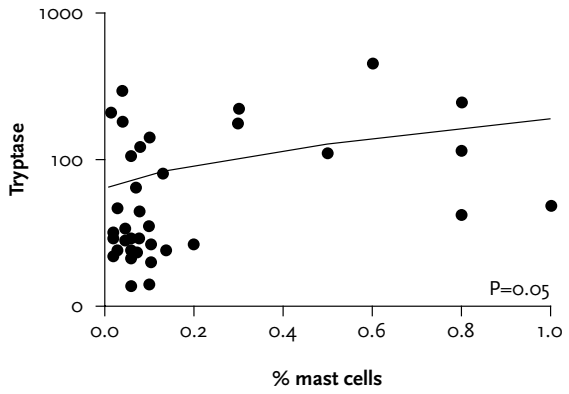
c-KIT mutations could be analysed in 34 SM patients and in 29 patients (85%) the D816V mutation was detected. c-KIT mutation analysis was not routinely performed in control subjects.

All but two SM patients had a serum tryptase level above 20 µg/ml. Serum tryptase was not routinely measured in control subjects, but two controls had an elevated serum tryptase although lower than 20 µg/ml.

A bone marrow biopsy showed multifocal clusters of aberrant mast cells in 30 SM patients (83.3%). Five patients (13.8%) had no clusters of aberrant mast cells. In one

patient the bone marrow biopsy was of insufficient quality for reliable interpretation.
The bone marrow smear showed spindle shaped mast cells in 33 SM patients (91.6%). As mentioned above, one control subject also had an increased number of aberrant mast cells. However, in the control group there was no active search for an increase in aberrant mast cells.
There was a weak association between serum tryptase and the percentage of aberrant mast cell in the bone marrow aspirate assessed by flow cytometry (*figure 1*).

**Figure 1.** *Association between % mast cells and serum tryptase (µg/ml)*




## DISCUSSION

The results of this study indicate that in most cases SM can accurately be diagnosed based solely on immunophenotyping of mast cells in a bone marrow aspirate. In a recent study by Akin *et al.* aberrant mast cells were also found in a bone marrow aspirate of patients with 'idiopathic anaphylaxis' initially not fulfilling sufficient criteria for the diagnosis of SM.[5] Nevertheless, all five patients with aberrant expression had at least one other minor criterion for SM suggesting that SM might be present in these patients as well. In fact, in three of these patients the diagnosis of SM could be confirmed based on the presence of three minor criteria.
Perhaps more importantly our results show that SM can be ruled out if immunophenotyping does not show aberrant expression of either CD2 or CD25 on CD117-positive mast cells.
This study has several drawbacks. First of all the number of controls is relatively small. Although this will not influence sensitivity and negative predictive value, specificity and positive predictive value may be overestimated. Furthermore, theoretically the results of this study may have been hampered by misclassification, as many of the diagnostic criteria for SM (serum tryptase, mutation analysis, bone marrow biopsy) could not be measured routinely in controls. However, this seems unlikely since SM is a very rare disease. Therefore the chance that a control subject is suffering from systemic mastocytosis is negligible.

Our study shows that immunophenotyping may be superior to other subcriteria in the diagnosis of SM. First, a bone marrow biopsy showing clusters of aberrant mast cells, the sole major criterion for SM, was negative in 13.8% of SM patients. This finding is in agreement with the results from Butterfield *et al.* who showed that in four out of 21 patients undergoing bilateral iliac crest biopsy, one of the biopsies was negative for SM.[6] Secondly, sensitivity and specificity of bone marrow smears are unknown but likely to be inferior to bone marrow biopsy and prone to be influenced by the experience of the laboratory technician.
Third, although none of our patients had a serum tryptase within the normal range, in two patients (6%) serum tryptase levels did not exceed 20 µg/ml, the minimum level to be regarded as a criterion for SM. In line with this observation, Sperr *et al.* reported that in eight out of 43 patients with SM, serum tryptase was below 20 µg/ml.[1] Furthermore, serum tryptase can also be elevated in various other conditions, including haematological malignancy and following an allergic reaction as shown in our control group.
Fourth, D816V mutations were found in 85% of patients screened for these mutations, but in five patients no such mutations were detected. These data are in agreement with the finding that the presence of the D816V mutation, found in more than 80% of patients, is a strong argument for the diagnosis of SM.[7,8] However, other c-KIT mutations (that are not looked for routinely) may occur as well.[1,9,10] In addition c-KIT mutations can also be found, although rarely, in patients with germ cell tumours and other neoplasms without coexisting SM.[11-13]

One might correctly argue that based on the data presented a diagnosis can also be made without immunophenotyping in the vast majority of patients (34 out of 36). The strength of immunophenotyping, however, lays in its high negative predictive value, obliviating the need to do further tests if negative.

## CONCLUSION

Based on the results of this study and given that immunophenotyping is fast and straightforward, we argue that immunophenotyping should routinely be used as first-line diagnostic tool to confirm or rule out SM.

## REFERENCES


1. Valent P, Akin C, Escribano L, et al. Standards and standardization in mastocytosis: consensus statements on diagnostics, treatment recommendations and response criteria. Eur J Clin Invest. 2007;37(6):435-53.
2. Pardanani A, Kimlinger T, Reeder T, Li CY, Tefferi A. Bone marrow mast cell immunophenotyping in adults with mast cell disease: a prospective study of 33 patients. Leuk Res. 2004;28(8):777-83.
3. Nunez-Lopez R, Escribano L, Schernthaner GH, et al. Overexpression of complement receptors and related antigens on the surface of bone marrow mast cells in patients with systemic mastocytosis. Br J Haematol. 2003;120(2):257-65.
4. Zhao W, Bueso-Ramos CE, Verstovsek S, Barkoh BA, Khitamy AA, Jones D. Quantitative profiling of codon 816 KIT mutations can aid in the classification of systemic mast cell disease. Leukemia. 2007;21(7):1574-6.
5. Akin C, Scott LM, Kocabas CN, et al. Demonstration of an aberrant mast-cell population with clonal markers in a subset of patients with 'idiopathic' anaphylaxis. Blood. 2007;110(7):2331-3.
6. Butterfield JH, Li CY. Bone marrow biopsies for the diagnosis of systemic mastocytosis: is one biopsy sufficient? Am J Clin Pathol. 2004;121(2):264-7.
7. Feger F, Ribadeau Dumas A, Leriche L, Valent P, Arock M. Kit and c-kit mutations in mastocytosis: a short overview with special reference to novel molecular and diagnostic concepts. Int Arch Allergy Immunol. 2002;127(2):110-4.
8. Tan A, Westerman D, McArthur GA, Lynch K, Waring P, Dobrovic A. Sensitive detection of KIT D816V in patients with mastocytosis. Clin Chem. 2006;52(12):2250-7.
9. Nakagomi N, Hirota S. Juxtamembrane-type c-kit gene mutation found in aggressive systemic mastocytosis induces imatinib-resistant constitutive KIT activation. Lab Invest. 2007;87(4):365-71.
10. Zhang LY, Smith ML, Schultheis B, et al. A novel K509I mutation of KIT identified in familial mastocytosis-in vitro and in vivo responsiveness to imatinib therapy. Leuk Res. 2006;30(4):373-8.
11. Sakuma Y, Sakurai S, Oguni S, Hironaka M, Saito K. Alterations of the c-kit gene in testicular germ cell tumors. Cancer Sci. 2003;94(6):486-91.
12. Biermann K, Goke F, Nettersheim D, et al. c-KIT is frequently mutated in bilateral germ cell tumours and down-regulated during progression from intratubular germ cell neoplasia to seminoma. J Pathol. 2007;213(3):311-8.
13. Kemmer K, Corless CL, Fletcher JA, et al. KIT mutations are common in testicular seminomas. Am J Pathol. 2004;164(1):305-13.