

Does the Aberrant Expression of CD2 and CD25 by Skin Mast Cells Truly Correlate with Systemic Involvement in Patients Presenting with Mastocytosis in the Skin?

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Key Words

Mastocytosis · Mast cells · CD2 · CD25 ·
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Abstract

Background: Neoplastic mast cells involving the bone marrow (BMMCs) of patients with mastocytosis display an aberrant expression of CD25 and/or CD2 antigens. The aim of this study was to determine the frequency of CD2 and CD25 expression on skin mast cells (sMCs) of patients with mastocytosis in the skin at the early stage of the disease. Furthermore, the usefulness of the phenotypic profile of sMCs for the diagnosis of systemic mastocytosis (SM) was evaluated.

Methods: The 52 adults included in the study were diagnosed with mastocytosis strictly according to the criteria of the World Health Organization. CD117, CD2 and CD25 antigen expression on sMCs was detected by immunohistochemistry. The presence of the *KIT* D816V mutation in the BM was analyzed using allele-specific PCR. **Results:** The presence of CD2- or CD25-positive sMCs was detected in 57.1% of cutaneous mastocytosis (CM) and 90.3% of SM cases ($p = 0.008$). In all mastocytosis patients, CD2 expression on sMCs was more frequent than CD25 expression (67.3 and 38.5%, respectively). Moreover, CD2 expression on sMCs was more

frequent in SM than in CM cases ($p = 0.02$). The presence of one of the aberrant sMC antigens was detected in 84.2% of patients with the *KIT* D816V mutation in the BM. A positive correlation between densities of CD25- and CD117-positive sMCs was found in SM patients ($r = 0.46$, $p = 0.009$). **Conclusions:** Although sMCs displayed immunoreactivity for one of the neoplastic antigens in the majority of SM patients, the aberrant CD2 and/or CD25 expression on sMCs is not as indicative of SM as the BMMC immunophenotype.

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Introduction

Mastocytosis is an uncommon and heterogeneous disease of bone marrow (BM)-derived hematopoietic progenitor cells that manifests with a broad spectrum of clinical and morphological appearances. Mastocytosis results from a clonal, neoplastic proliferation of morphologically and immunophenotypically abnormal mast cells (MCs) [1–3]. The disease is characterized by the abnormal accumulation of MCs in various organs. The BM, skin, liv-

M.L. and A.Ż. shared equal contribution.

er, spleen, lymph nodes, and gastrointestinal tract are commonly affected. The World Health Organization (WHO) classified mastocytosis as a one of eight subcategories of myeloproliferative neoplasms [4, 5]. Mastocytosis may be limited to the skin (cutaneous mastocytosis, CM) or it may also involve extracutaneous sites (systemic mastocytosis, SM) or mastocytosis in the skin (MIS) coexisting with internal organ pathology (SM with MIS) [6–8]. MIS is an initial diagnosis which requires further diagnostic steps leading to the final differentiation between CM and SM [7]. In adults, MIS is often the first clinical manifestation of indolent SM (ISM). Poor prognosis forms of SM such as aggressive SM (ASM), SM associated with clonal hematological non-MC lineage disease (SM-AHNMD) and mast cell leukemia (MCL) usually occur without MIS [4]. According to the WHO recommendations, the major criterion of SM is the histological demonstration of multifocal compact MC infiltrates in BM (>15 MCs in aggregate) or other extracutaneous tissues. The minor criteria are the following: (1) *KIT* D816V mutation in extracutaneous organ(s), (2) MCs in BM expressing CD2 or/and CD25, (3) MCs in BM or other extracutaneous organ(s) showing an atypical morphology and (4) total tryptase level in serum exceeding 20 ng/ml (does not count in patients with SM-AHNMD) [4, 5]. The final diagnosis of SM may be rendered if one major and one minor criterion or three minor criteria are fulfilled [1–5].

Somatic gain-of-function mutations within the coding sequence of the *KIT* gene are detected in patients with mastocytosis. The most frequent of these is a point mutation D816V of *KIT* exon 17. This mutation has been detected in up to 85% of all SM patients, irrespective of the subtype [2, 9, 10]. *KIT* (CD117) is a type III tyrosine kinase receptor. The interaction between *KIT* and its ligand stem cell factor plays a crucial role in the regulation of MC proliferation, maturation, adhesion, chemotaxis, and survival. D816V mutation leads to ligand-independent, constitutive activation and autophosphorylation of the *KIT* receptor and, therefore, appears to be central to the pathogenesis of SM [2, 4, 9].

Recent data indicate that MCs are heterogeneous in terms of cell surface antigen expression, which is considered to be related to MC plasticity, disease-associated factors and the maturation stage of the cells [11]. The receptor *KIT* (CD117) is expressed on all types of MCs and MC-committed progenitor cells, regardless of their maturation stage and activation status. Thus, it facilitates the detection of MCs in different tissues [11–13]. In the majority of SM patients, the aberrant expression of CD2

(LFA-2) and CD25 (IL-2RA) on neoplastic MCs in the BM (BMMCs) is observed. These are 'lymphoid-associated' antigens that are not identified on the normal MCs [11, 14]. Thus, CD2 and CD25 expression on MCs is regarded as a minor diagnostic WHO criterion of SM. CD25 is considered as a more sensitive parameter that is typically expressed on the neoplastic MCs and therefore recommended as the marker of choice [15]. In addition, multiple recent and past studies have described the BMMC immunophenotype in detail [11–16]. In contrast, little is known about the immunophenotypic profile of skin MCs (sMCs) in patients with mastocytosis [17–20]. It has been reported that sMCs in mastocytosis patients may coexpress CD25 and CD4 but do not express CD2. However, it has to be stressed that those studies were based on small groups of patients [17, 19, 20]. Furthermore, the frequency of CD2 and CD25 expression on sMCs has never been assessed in CM and SM patients, who were diagnosed strictly according to the WHO criteria.

In the current study, the frequency of CD2 and CD25 expression on sMCs was analyzed in a group of patients with MIS. Skin biopsy is a routinely performed procedure in patients with suspected MIS. Therefore, we decided to evaluate whether the immunohistochemical examination of CD2 and CD25 expression on sMCs could be a useful criterion for the diagnosis of underlying SM in patients at the early stage of the disease. Moreover, we assessed the relationship between the aberrant immunoreactivity of sMCs and the presence of the *KIT* D816V mutation in the BM of patients with MIS.

Materials and Methods

The study group consisted of 52 adult patients (aged 18 years and older): 36 females and 16 males with a mean age of 43.4 years (range: 18–77 years). They were diagnosed in the Gdańsk Mastocytosis Center, which is a reference site for the whole Polish population of mastocytosis patients. All patients gave written informed consent for the immunohistochemical examination of skin samples and the other diagnostic procedures. The study was approved by the local ethics committee. All patients presented a maculopapular form of skin disease and in all individuals MIS was confirmed by histopathological examination of the skin samples. Skin biopsy was performed at the first visit to the center. In the studied group of patients, the medical history of MIS was no longer than 3 years. All patients were checked for SM by using the WHO criteria. CM was diagnosed in 21 (40.4%) cases whereas SM was confirmed in 31 (59.6%) patients. BM biopsy revealed compact infiltrates of MCs in 19 (61.3%) SM patients. In a group of 12 (38.7%) SM patients the diagnosis was based on minor criteria. Flow cytometry revealed that BMMCs expressed CD2 and CD25 in 46 (84.5%) and

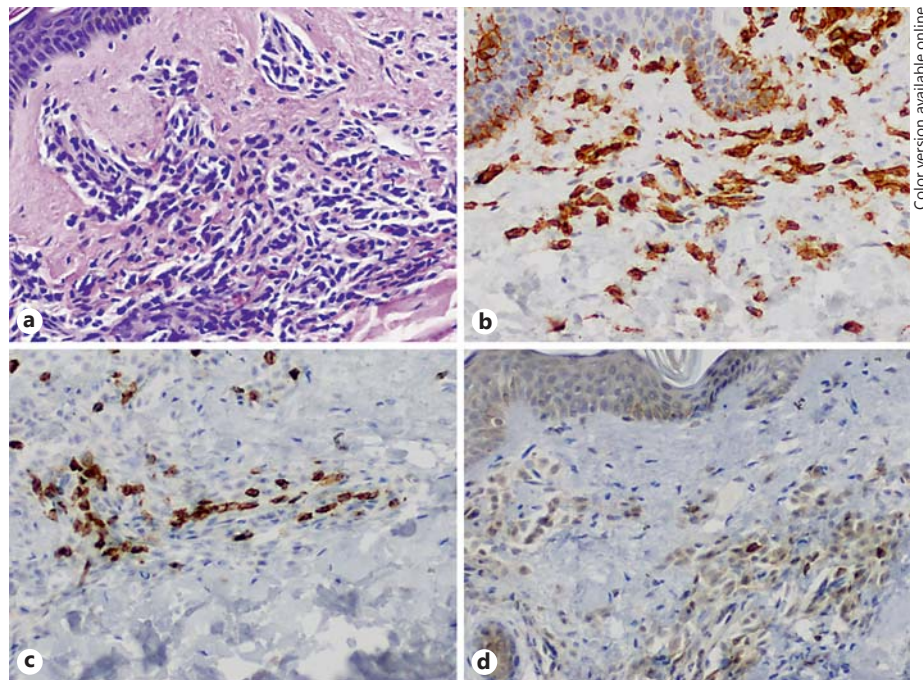


Fig. 1. MIS of a patient with SM. **a** A dense MC infiltrate in the upper dermis. HE stain. **b** Expression of CD117 on sMCs. **c** Immunohistochemical detection of CD2 on sMCs. **d** Immunohistochemical detection of CD25 on sMCs.

48 (92.3%) cases, respectively. Atypical BMMC morphology was observed in 40 (76.9%) cases and persistent elevation of baseline serum tryptase levels was detected in 30 (57.7%) patients. Mutational analysis was performed using the allele-specific PCR. In total, D816V mutation was detected in 38 (73%) BM biopsies, of which 8 (38.1%) were taken from CM patients and 30 (96.7%) from SM patients. The immunohistochemical examination of CD117, CD2 and CD25 on sMCs was determined in all of the 52 patients included to the study. Skin was obtained from the trunk or the thigh, fixed in 10% buffered formaldehyde solution and embedded in paraffin wax. Immunohistochemistry was performed on 3- μ m sections using the immunoperoxidase staining technique. Endogenous peroxidase was blocked by H_2O_2 . The slides were incubated with the polyclonal antibody against CD117 (code A4502, 1:400 Dako, Glostrup, Denmark), monoclonal antibody against CD2 (code NCL-L-CD2-271, clone AB75, 1:600; Novocastra, Nussloch, Germany) and monoclonal antibody against CD25 (code NCL-CD25-305, clone 4C9, 1:400; Novocastra) for 1 h and then washed and incubated with the secondary antibody for 30 min (EnVision Flex, Dako for CD117 and Novolink, Novocastra for CD2 and CD25). The slides were washed and antibody localization was effected using a peroxidase reaction with 3,3'-diaminobenzidine tetrahydrochloride (Dako) as chromogen. The slides were then washed, counterstained, dehydrated, and mounted. Appropriate positive and negative controls were used. MC density was defined by the number of cells per high-power field using a $\times 40$ objective and a $\times 10$ ocular lens (field size: 0.237 mm²). sMCs were identified as round cells with ovoid nuclei and abundant pale cytoplasm or as spindle/fusiform cells with elongated nuclei (fig. 1a) which expressed CD117 (fig. 1b). Immunoreactivity for CD2 (fig. 1c) and CD25 (fig. 1d) in sMCs showed a membrane-cytoplasmic staining pattern. A case was defined as having CD2-positive and/or CD25-positive sMCs when at least 2 CD2-positive and/or CD25-positive

MCs were found in 3 high-power fields. Distinguishing between CD2- and CD25-positive MCs and T cells was based on the morphological features of these cells and weaker CD2 and CD25 immunostaining of MCs compared with the strong immunoreactivity of T cells. Furthermore, MCs were larger than T cells, which were characterized by scanty cytoplasm and rounded nuclei. Control normal skin was obtained from 23 adult patients who had not suffered from any skin disease and who had undergone surgery for clinical reasons other than mastocytosis.

The Mann-Whitney U test was used to assess the association between categorical variables. Fisher's exact probability test was carried out for frequency analysis. Correlation coefficients were determined using Spearman's rank correlation test. p values below 0.05 were considered to be statistically significant. STATISTICA 10.0 PL (StatSoft, Inc.) software was applied.

Results

In the studied group of 52 patients with mastocytosis, the expression of CD2 and CD25 on sMCs was detected in 35 (67.3%) and 20 (38.5%) individuals, respectively. The expression of at least one aberrant antigen was observed in the majority of cases ($n = 40$, 76.9%). Both aberrant antigens were displayed in 13 (25%) of the mastocytosis patients. Finally, no aberrant sMC immunophenotype was detected in 12 (23.1%) individuals.

CD2-positive sMCs were detected in 47.6% of CM cases and in 80.6% of SM cases, whereas CD25 expression on sMCs of CM and SM patients was 23.8 and 41.9%, respec-

Table 1. Frequency of CD2- and CD25-positive MCs in the skin of CM and SM patients and in control skin

Skin MC phenotype	Control (n = 23)		CM (n = 21)		SM (n = 31)		P (CM vs. SM)
	n	%	n	%	n	%	
CD2	0	0	10	47.6	25	80.6	0.02
CD25	0	0	5	23.8	15	41.9	0.24
CD2 and CD25	0	0	3	14.3	10	32.3	0.20
CD2 or CD25	0	0	12	57.1	28	90.3	0.008

Table 2. Comparison of MC density in the skin of CM and SM patients

Skin MC phenotype	Density of skin infiltration (MCs/HPF)						p
	CM			SM			
	median	LQ	UQ	median	LQ	UQ	
CD117 (all MCs)	69	56	91	78	45	176	0.40
CD2	0	0	18	8	0.7	30	0.12
CD25	0	0	0	0	0	3	0.36

LQ = Lower quartile; UQ = upper quartile; HPF = high-power field.

tively (table 1). The expression of CD2 on sMCs was significantly more frequent in SM than in CM cases ($p = 0.02$). The aberrant expression of both CD2 and CD25 on sMCs was found in 14.3% of CM patients and in 32.3% of SM patients. The presence of one of the aberrant sMC surface antigens, CD2 or CD25, was detected in 57.1% of CM and 90.3% of SM cases and it was significantly more frequent in SM than in CM ($p = 0.008$). In all patients with CD2-positive and/or CD25-positive sMCs the immunoreactivity for CD2 and/or CD25 was found in BMNCs. The comparison of the density of CD117-, CD2- and CD25-positive MCs in skin infiltrates revealed no significant differences between CM and SM cases (table 2).

In a group of patients carrying the *KIT* D816V mutation in the BM, the presence of CD2 or CD25 on sMCs was detected in 84.2% of cases. Moreover, in this group the expression of CD2 was more frequent than CD25 (table 3). Statistical analysis revealed no significant differences in the frequency of CD2-positive and/or CD25-positive sMCs in patients with or without the *KIT* D816V

Table 3. Frequency of CD2- and CD25-positive MCs in the skin of patients with and without *KIT* D816V mutation in BM

Skin MC phenotype	<i>KIT</i> D816V mutation in BM				p
	negative (n = 14)		positive (n = 38)		
	n	%	n	%	
CD2	7	50	28	73.7	0.18
CD25	4	28.6	14	36.8	0.75
CD2 and CD25	3	21.4	10	26.3	1.0
CD2 or CD25	8	57.1	32	84.2	0.06

Table 4. Comparison of MC density in the skin of mastocytosis patients with and without *KIT* D816V mutation in BM

Skin MC phenotype	Density of skin infiltration (MCs/HPF)						p
	negative <i>KIT</i> D816V mutation in BM			positive <i>KIT</i> D816V mutation in BM			
	median	LQ	UQ	median	LQ	UQ	
CD117 (all MCs)	77.8	56.7	158.7	75.5	42.7	141.3	0.57
CD2	1.5	0	30	6.7	0	18	0.59
CD25	0	0	1.5	0	0	3	0.74

LQ = Lower quartile; UQ = upper quartile; HPF = high-power field.

mutation in the BM. In addition, no significant difference in the density of CD117-, CD2- and CD25-positive sMCs was observed in these two groups of patients (table 4).

Further statistical analysis revealed a positive correlation between the density of CD25- and CD117-positive sMCs in the skin infiltrates of all studied patients ($r = 0.41$, $p = 0.002$), including SM patients ($r = 0.46$, $p = 0.009$), individuals bearing the *KIT* D816V mutation ($r = 0.40$, $p = 0.012$) and patients who met the major criterion of SM ($r = 0.53$, $p = 0.018$). We also found a correlation between CD2- and CD117-positive sMC density in analog analyses, but the result was not statistically significant.

Discussion

SM with or without skin involvement is the predominating form of mastocytosis in adult-onset patients [1, 4, 7]. In our study group SM also occurred more frequently than CM. Nevertheless, the mastocytosis was limited to

the skin in 40.4% of patients presenting MIS. It has to be underlined that cytological, histological, immunohistochemical, and genetic examinations according to the WHO-defined SM criteria were performed in all participants. The high frequency of CM in our study group of patients could be due to the fact that a substantial number of subjects were referred to our center by dermatologists and did not present other mastocytosis symptoms except for skin lesions and mediator-related symptoms. Moreover, the patients enrolled in the study were diagnosed at the initial stage of the disease. It has been reported that the presence of dense multifocal BMMC aggregates may be missed in a significant proportion of SM cases at the early stages of the disease [21]. Therefore, we decided to assess the usefulness of sMC immunoreactivity for CD2 and/or CD25 as a predicting factor for SM in MIS patients at the early stage of mastocytosis.

It is commonly accepted that the abnormal expression of CD2 and CD25 by BMMCs is specific for SM [14, 21–25]. More recent data has shown that the isolated CD25 expression by BMMCs is more informative than the co-expression of CD25 and CD2 [15, 23]. It has been shown that in all patients with CD25-positive BMMCs, the *KIT* D816V mutation was detected in BM tissue samples [15]. Nowadays, CD25 expression by MCs and the presence of the *KIT* D816V mutation in the BM are considered to be typical features of clonal MC proliferation [21, 23, 24]. Recent studies have indicated that childhood-onset mastocytosis, represented predominantly by CM, is also clonal in nature and is associated with various activating *KIT* mutations [4, 26, 27]. Therefore, it was essential to find out whether sMCs in CM and SM patients also display an aberrant immunophenotype in routinely processed skin biopsy specimens.

Our immunohistochemical study indicated that sMCs aberrantly express CD2 and/or CD25 in a considerable subset of mastocytosis patients. At least one of these antigens was detected in 57% of CM cases and in 90% of SM cases. Nevertheless, in 23% of MIS patients neither CD2 nor CD25 expression was detected. Thus, it could be postulated that the frequency of CD2-positive and/or CD25-positive BMMCs is higher compared with sMC immunoreactivity in MIS patients.

As assessed by immunohistochemistry, sMCs in patients with mastocytosis showed a more common expression of CD2 than CD25. CD2-positive sMCs were detected in almost 50% of CM cases and in approximately 80% of SM cases. Moreover, the aberrant expression of CD2 on sMCs was significantly more frequent in SM than in CM cases. However, CM and SM patients did not differ

with respect to the density of CD2-positive MCs in skin infiltrates. CD2 is a ligand of CD58 (LFA3) and the CD2-CD58 interaction may contribute to cluster formation of BMMCs in SM [28]. It could be postulated that the expression of CD2 on neoplastic BMMCs has a lower diagnostic value compared with CD25 immunoreactivity. Sotlar et al. [15] reported that CD2-positive BMMCs were detectable with a frequency of 81% in ISM patients, whereas in ASM it was only 29%. BMMC immunoreactivity for CD2 is typical for ISM and smoldering SM, which are usually associated with MIS [11, 21, 25, 29]. In addition, it was speculated that the loss of CD2 during disease progression may contribute to the leukemic spread of MCs [11, 28]. Thus, it is not surprising that BMMCs in ASM and MCL often display lower levels of CD2 compared with BMMCs in ISM [11, 15]. Interestingly, in the majority of ASM and MCL cases skin involvement is absent [21, 25, 29].

In the current study, CD25 expression on sMCs was a rare finding. It was detected in less than 25% of CM and in less than 50% of SM cases. CD25 is the alpha chain of the IL-2 receptor. This antigen is usually expressed on BMMCs in SM and is considered to be a key marker of neoplastic BMMCs [11, 15, 23]. Here, we present data indicating an apparent difference between CD25 expression on sMCs in CM and SM (23.8 vs. 41.9%, respectively); however, the result was not statistically significant. Moreover, the density of CD25-positive MCs was not significantly different in the skin infiltrates of CM and SM patients. Our results are in contrast with those reported by Hollmann et al. [17], who concluded that CD25 expression on sMCs in adult patients presenting with urticaria pigmentosa was predictive for SM. Nevertheless, the authors reported that only half of all patients in their study underwent BM biopsy. According to recent consensus statements on mastocytosis diagnostics, histological BM analyses, including immunohistochemistry and molecular genetics, are required in all adult patients [1, 4, 7]. In practice, the diagnostic procedure in MIS patients should begin with a BM examination since this site is almost invariably involved in adult mastocytosis and allows the detection of associated hematological neoplasms.

Sotlar et al. [15] revealed that all patients with CD25-positive BMMCs presented the *KIT* D816V mutation. A recent study concerning BMMC immunoreactivity showed that the aberrant expression of CD25 with FcεRI^{lo}, FSC^{lo}, SCC^{lo}, and CD45^{lo} on BMMCs is associated with multilineage involvement by the *KIT* D816V mutation, regardless of the subtype of SM [30]. However, according to our results the same is not true for the sMC immuno-

phenotype. We found that there was no significant difference in the frequency of CD2 and CD25 expression on sMCs in a group of patients positive or negative for the *KIT* D816V mutation. Interestingly, there was also no significant difference in respect to the density of CD25- and CD2-positive sMCs between patients with or without the *KIT* D816V mutation in the BM. We found that the majority of patients who carried the *KIT* D816V mutation displayed the expression of at least one of these antigens on sMCs (84.2%). Interestingly, in 14 MIS patients included in our study the *KIT* D816V mutation was not detected. It is possible that in a certain subgroup of these patients the occurrence of mastocytosis was associated with another, less common, somatic *KIT* mutation, for instance such as that previously identified in adult mastocytosis patients [4, 26, 31].

In our study skin biopsy was obtained only from the trunk and the thigh. These locations are characterized by a similar density of sMCs to avoid differences associated with various MC distribution in adult skin [32]. There was no significant difference in the density of CD117-positive sMCs between CM and SM patients and between patients with or without the *KIT* D816V mutation in the BM. Interestingly, a correlation between CD117- and CD25-positive sMC density was found in SM patients, in those carrying the *KIT* D816V mutation in the BM and in those with multifocal, compact MC infiltrates in the BM. The clinical implications of these findings are currently unclear. Nevertheless, a positive correlation between the density of CD117- and CD25-positive MCs in the skin infiltrates of mastocytosis patients who met the criteria of systemic involvement and the lack of a similar correlation

in the skin of CM patients suggest that the aberrant expression of CD25 on sMCs has some diagnostic value for SM. It is noteworthy that, for all patients included in the study, skin biopsy and immunostaining of sMCs were performed during the initial diagnostic period. Furthermore, all patients are being followed up in our center. Thus, it can be speculated that a long-term observation of the disease course in these patients will allow us to assess whether sMC immunoreactivity for CD2 and/or CD25 is useful in the prediction of underlying SM in the early stage of the disease.

We conclude that sMCs in patients with mastocytosis present a heterogeneous phenotype. It is noteworthy that the majority of SM patients and MIS individuals with the *KIT* D816V mutation displayed the expression of at least one of the aberrant antigens on sMCs (90 and 84%, respectively). We also found that sMC immunoreactivity for one of the neoplastic antigens was significantly more frequent in SM than in CM patients. Nevertheless, CD2 and/or CD25 expression on sMCs is not such an unequivocal marker of SM as the BMMC phenotype.

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