Cutaneous Lesions of Mastocytosis: Mast Cell Count, Morphology, and Immunomolecular Phenotype

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Abstract: Mastocytosis is a condition characterized by accumulation of clonal mast cells (MCs) that often involves the skin. Pathologists are often challenged with skin biopsies with a question of cutaneous lesions of mastocytosis (CLM) including cutaneous mastocytosis, mastocytosis in the skin, or systemic mastocytosis. The histopathological criteria for CLM remain poorly defined due to heterogeneity of the published literature and the lack of comparative prospective studies. MC count is greatly influenced by detection and counting techniques, criteria for viable MCs used, anatomical location biopsied, and the dermal level that is analyzed. Although MC numbers in CLM can be significantly higher compared with healthy controls and a patient with other inflammatory skin diseases, in some instances, considerable overlap exists. Based on the largest studies published, it is suggested that a number of MCs between 75 and 250 MCs/mm^2 are a range in which CLM should be considered and, above 250 MC/mm^2, a diagnosis of CLM can be made. A recent study showed a high specificity of >95% of a MC count >139 MC/mm^2 compared with patients with other inflammatory skin diseases. Noteworthy, the total number and percentage of MCs is significantly higher in children compared with adults, particularly in polymorphic maculopapular cutaneous mastocytosis. In difficult cases, ancillary techniques such as D816V mutation analysis on formalin-fixed paraffin-embedded tissue have a high sensitivity and specificity. There is no enough evidence that immunohistochemistry of CD25, CD2, or CD30 has any additional value in the diagnosis, subtyping, or clinical course of mastocytosis.

Key Words: mastocytosis, mast cell, skin, biopsy, CD2, CD25, CD30

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INTRODUCTION

Mastocytosis is a condition characterized by accumulation of clonal mast cells (MCs) that mainly affects the skin. The World Health Organization (WHO) classification defines 3 major categories: cutaneous mastocytosis (CM), systemic mastocytosis (SM), and localized extracutaneous MC neoplasm. SM is defined by the presence of neoplastic MCs in at least one extracutaneous organ, most often bone marrow (BM) and more rarely liver, spleen, lymph nodes, or gastrointestinal tract.[1] In CM, MCs are restricted to the skin and this is predominantly found in children. CM includes maculopapular CM (MPCM, formerly known as urticaria pigmentosa), diffuse CM, and cutaneous mastocytoma. Telangiectasia macularis eruptiva perstans was not included as a separate entity anymore in the 2017 WHO classification of tumors of hematopoietic and lymphoid tissues.[2] MPCM can be subdivided into polymorphic MPCM and monomorphic MPCM, of which the latter has been associated with progression to chronic mastocytosis and SM in children.[1] SM is diagnosed by an increased MC number in at least one extracutaneous organ, and BM examination is virtually always mandatory for a definitive diagnosis of SM. Until complete staging has been performed, a provisional diagnosis of "mastocytosis in the skin" (MIS) can be made. Cutaneous involvement of SM is seen in most of the cases.[3]

Pathologists are often challenged with a question of cutaneous lesions of mastocytosis (CLM), which could either be CM, MIS, or SM. For the purpose of this review, we introduce the term CLM, which should not be confused with MIS, which is a provisional diagnosis pending BM examination. In many cases, the histopathological diagnosis is straightforward, such as in most cases of mastocytoma in which histology reveals dense sheets of MCs. However, this can be more challenging for certain types of MPCM, particularly in cases previously classified as telangiectasia macularis eruptiva perstans. In these cases, it is up to the pathologist to differentiate CLM from normal skin or other inflammatory dermatoses. In these cases, MC count, morphology, distribution, immunophenotype, and molecular analysis might be helpful ancillary tools to establish a more accurate diagnosis. However, the histopathological criteria for CLM remain poorly defined due to heterogeneity of the published literature and the lack of comparative prospective studies. In this review, we will outline the literature about CLM and we critically evaluate the added value of the previously described features.

MAST CELL NUMBER IN SKIN

In normal healthy skin, no definite reference values for the numbers of MCs exists. Establishing a firm reference range is made difficult by several factors:

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TABLE 1. Mast Cell Count in Normal Skin, Inflammatory Skin Diseases and in Cutaneous Lesions of Mastocytosis as Reported in the Literature

Reference	Population	Biopsy Location	Technique	Dermal Level Counted	MC Number (Mean, Unless Otherwise Specified)
Cowen, 1979[28]	Healthy volunteers (n = 9) Adults	Lower arm	Basic fuchsin and alkaline methylene blue	Transdermal	43.58 ± 6.06 (SE) MC/mm^2
		Upper arm			50.47 ± 4.90 (SE) MC/mm^2
Rosen, 1987[29]	Autopsies (n = 55) Children and adults	Upper thigh (non–sun-exposed)	Leder, Giemsa	Papillary	60 (range 10–151) MC/mm^2
		Neck (sun-exposed)			65 (range 16–122) MC/mm^2
Garriga, 1987[30]	Healthy volunteers (n = 9) Adults	Inner aspect of the lower arm	Toluidine blue	Transdermal	38 ± 4 (SE) MC/mm^2
	Unexplained anaphylaxis/flushing (n = 9) Adults				72 ± 13 (SE) MC/mm^2
	Systemic mastocytosis (n = 12) Adults				Lesional: 721 ± 178 (SE) MC/mm^2
					Non-lesional: 184 ± 38 (SE) MC/mm^2
	Urticaria pigmentosa (n = 6) Adults				Lesional: 597 ± 278 (SE) MC/mm^2
					Nonlesional: 168 ± 74 (SE) MC/mm^2
Wilkinson, 1992[31]	Healthy volunteers (n = 10) Age not mentioned	Not mentioned	Leder	Transdermal	54 ± 7 (SE) MC/mm^2 *
	Inflammatory dermatoses (n = 50) Age unknown				43 ± 5 (SE) MC/mm^2 *
	CLM (n = 30) Adults				382 ± 28 (SE) MC/mm^2 *
Sweet, 1996[11]	Normal skin controls (n = 11) Age not mentioned	Upper and lower extremities, abdomen, trunk, neck	Giemsa	Transdermal	8.2 median (range 2–16.1) MC/mm^2
	Urticaria (n = 20) Adults				10.1 median (range 2.4–27.2) MC/mm^2
	Dermal hypersensitivity reaction (n = 19) Adults				9.3 median (range 1.8–18.3) MC/mm^2
	Urticaria pigmentosa (nodular) (n = 7) Children and adults				465 median (range 278–1108) MC/mm^2
	Urticaria pigmentosa (perivascular) (n = 15) Children and adults				89 median (range 46–327) MC/mm^2
Weber, 2003[10]	Normal skin, excess skin removed during dermatological surgery performed for diagnostic or cosmetic reasons (n = 150) Children and adults	Whole body (mapping)	Toluidine blue	Transdermal	20–60 (minimum - maximum) MC/mm^2
Grimbaldeston, 2003[7]	Healthy volunteers (n = 19) Adults	Buttock	Antihistamine and anti-CD117	Transdermal	35 median (range 12–47) MC/mm^2
		Inner arm			39 median (range 21–57) MC/mm^2
		Shoulder			33 median (range 16–65) MC/mm^2
		Hand			64 median (range 31–110) MC/mm^2

TABLE 1. Mast Cell Count in Normal Skin, Inflammatory Skin Diseases and in Cutaneous Lesions of Mastocytosis as Reported in the Literature (*Continued*)

Reference	Population	Biopsy Location	Technique	Dermal Level Counted	MC Number (Mean, Unless Otherwise Specified)
Janssens, 2005[9]	Normal skin, excess skin removed during dermatological surgery performed for diagnostic reasons (n = 141)	Proximal (trunk, upper arm, and upper leg)	Antitryptase	Transdermal	77 ± 33.6 (SD) MC/mm^2
		Distal (lower arm, lower leg)			108.2 ± 41.4 (SD) MC/mm^2
	Adults				
	CLM (n = 14)	Distal (lower arm and lower leg)			821 ± 582 MC/mm^2 (range 78–2409)
	Children and adults				
Kim, 2008[8]	Healthy volunteers (n = 22)	Buttocks (non–sun-exposed)	Antitryptase and antichymase	Transdermal	69.8 ± 3.9 MC/mm^2
	Adults				
		Face (sun-exposed)			109.0 ± 8.5 MC/mm^2
Bretterklieber, 2015[32]	Autopsies (n = 10)	Neck	Antitryptase and anti-CD117	Not mentioned	39 (range 14–62) MC/mm^2
	Children and adults				
	Urticaria (n = 7)	Not mentioned			84 (range 68–104) MC/mm^2
	Adults				
	Anaphylaxis (n = 14)				93.1 (range 66–124) MC/mm^2
	Adults				
	Systemic mastocytosis (n = 5)	Normal neck skin			124 (range 84–178) MC/mm^2
	Adults				
Gebhard, 2020[6]	Controls (n = 36: Eczema n = 10, pruritus n = 7, urticaria n = 10, tumor margin n = 10)	Thigh Upper trunk	CD117 Tryptase	Transdermal	CD117: 7 median (range 0–70) Tryptase: 32 median (range 0–92)
	Adults				
	CLM (n = 47: 32 adults (15ISM and 17 MIS) and 1 child with MPCM and 14 mastocytoma (all children))				CD117: 137 median (range 9–414) Tryptase: 136 median (range 0–342)
	Children and adults				
Drabent, 2021[12]	Controls (n = 22, tumor margin)	Not mentioned	CD117	Areas with highest MC density	25.1 (SD 10.3)
	Age not mentioned				
	Inflammatory controls (n = 63: Eczema/atopic dermatitis, lichen planus, prurigo, bullous pemphigoid, pityriasis lichenoides, and psoriasis)				38.3 (SD 24.8)
	Age not mentioned				
	Mastocytosis (n = 103) including SM and CM				202 (SD 160)
	Children and adults				
Hermans, 2022[15]	pMPCM (n = 8)	Not mentioned	CD117 Tryptase	Transdermal	2145 + 546 SE MC/mm^2
	Children				
	mMPCM (n = 10)				2084 + 719 SE MC/mm^2
	Children				
	DCM (n = 3)				3611 + 1139 SE MC/mm^2
	Children				
	SM (n = 7) adults				374 + 60 SE MC/mm^2

*Values only given for superficial dermis.

First, although an experienced dermatopathologist can recognize MCs in H&E sections, in most cases, ancillary techniques are mandatory to visualize MCs. In the beginning, histochemical stains were used to visualize MCs such as Leder, methylene blue-basic fuchsin, toluidine blue, astra blue, and May–Grunwald–Giemsa. Later on, the gold standard changed to more specific immunohistochemical stainings by monoclonal antibodies against chymase, tryptase, and/or CD117.[4,5] Positive stainings of CD117 and tryptase have been shown to correlate well.[5,6]

Second, MCs vary greatly in numbers across different anatomical locations. The number of MCs is significantly higher at the lower legs and feet, lower arms and hands, and in the face. The number of MCs is also higher when counted in the upper dermis, in the face, and in sun-exposed areas.[6–10] Furthermore, MCs are normally found surrounding adnexal structures and dermal nerves and should therefore be avoided when counting MCs. There is also a great variability due to the criteria by which MCs are identified: Although some authors only count MCs when nuclei are present, others only include nucleated MC when a certain number of granules are found in cytoplasm or with a fully stained membrane.[10–12]

Third, MC count is greatly influenced by the counting method because most pathologists determine the number of MCs per high-power field (HPF). HPF varies between microscopes, and therefore, it is essential to assess the MC number per mm^2. In addition, counting techniques vary from histomorphometry to conventional eyeballing methods under the microscope. Because no uniform studies exist and different studies used HPF and/or mm^2, it makes it very difficult to compare with earlier published studies. Based on the 2 largest studies published so far by Janssen et al and Weber et al in normal skin, it is suggested that a figure up to 75 MC/mm^2 should be considered as normal. A number more than 250 MC/mm^2 should be regarded as abnormal and most likely confirms a diagnosis of mastocytosis. A number of MCs between 75 and 250 MCs/mm^2 are a range in which mastocytosis should be considered, thereby taking into account that an individual approach is always necessary and clinical–pathological correlation is mandatory.[9,10] Table 1 summarizes the most important studies so far on normal skin and CLM. The table only summarizes studies that reported the number of MC in mm^2 and not per HPF. It shows a wide variation from mean/median of 121–821 MC/mm^2 in CLM, with ranges overlapping with "normal MC number" of <75 MC/mm^2. No large prospective comparative studies have investigated the difference in the number of MCs between different types of mastocytosis (SM vs. adult CM and pediatric CM).

Although the previously cited studies are interesting, most of these studies have focused on the number of MCs in mastocytosis versus normal skin. However, in daily clinical practice, pathologists are confronted with a differential diagnosis of mastocytosis versus other inflammatory dermatoses. Table 1 summarizes the number of MC found per mm^2, including studies that compared mastocytosis with inflammatory skin conditions and normal skin. Altogether, these studies demonstrated that a higher number of MC can be found in mastocytosis (reported median 89 MC/mm^2 or mean 721 MC/mm^2) compared with inflamed skin (reported median 7 MC/mm^2 or mean 93 MC/mm^2). Noteworthy, a recent retrospective study by Gebhard et al[6], 47 patients with MPCM, 32 adults (15 ISM and 17 MIS) and 1 child, and 14 children with mastocytoma were compared with controls and analyzed for different parameters (MC number, morphology, distribution, immunophenotype, and mutational status). An optimized cutoff of 62 MC/mm^2 in the upper dermis (directly subepidermal, based on the published photographs: papillary dermal and superficial reticular dermal) was determined to have a sensitivity of 91% and a specificity of 92% compared with controls. This number is more or less comparable with the cutoff as suggested by Janssen et al and Weber et al analyzing normal skin. An increased specificity to $>95\%$ (with loss of sensitivity to 78.1%) was found for cutoff of 139 MC/mm^2 in the upper dermis. Figure 1 shows an example of a skin biopsy from normal skin (A) with a density of 85 MC/mm^2 in the upper dermis compared with 620 MC/mm^2 in a patient with CLM (B).

MAST CELL MORPHOLOGY, DISTRIBUTION, AND OTHER SKIN CHANGES

MCs of patient with CLM can show atypical indented bilobated nuclei and numerous elongated cytoplasmic projections compared with MCs in normal skin.[13] MC size in MPCM in adults has also been found marginally but significantly larger than compared with controls with inflammatory skin conditions.[6] Furthermore, MCs in lesional skin from patients with SM demonstrated an increased cytoplasmic area, a larger nuclear size, and diameter of granules compared with skin MC from non-SM patients.[14] MCs in the skin of mastocytosis can be spindle-shaped, spherical but also bilobated and pleomorphic. Spindle shape is not a discriminative feature between CLM and inflamed controls.[6] None of these cytonuclear changes have been associated with the clinical subtype or progression from CM to SM so far.[15]

Concerning the overall distribution of MC in the skin of mastocytosis, Drabent et al[12] have recently made a comparison between mastocytosis (n = 103) and other inflammatory dermatoses (n = 63) and found that a sheet-like and subepidermal distribution are specific for mastocytosis compared with other inflammatory dermatoses. The most significant finding of their study was the *percentage* of MCs compared with the total number of inflammatory cells (mostly lymphocytes), not the absolute MC number. A percentage of more than 40% was highly specific for mastocytosis but lacked sensitivity, particularly in adults.

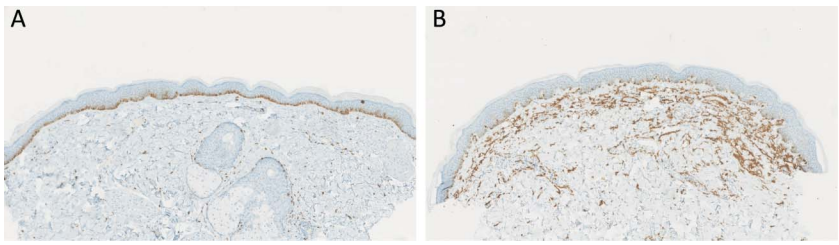

FIGURE 1. Examples of MCs visualized by CD117 immunohistochemistry in normal skin (A) with a density of 85 MC/mm^2 in the upper dermis compared with 620 MC/mm^2 in a patient with CLM (B).

When the histopathological features are suggestive or diagnostic for CLM, the dermatopathologist could potentially help to differentiate the different types of CLM skin by evaluation of the dermal distribution pattern. Wolf et al recognized 4 distribution patterns, illustrated in Figure 2, ranging from (A) perivascular in the papillary body and upper dermis, (B) sheet-like within the papillary body and upper reticular dermis, (C) interstitial, and (D) nodular.[16] However, they concluded that the distribution pattern does not allow predicting the course of the disease, nor was it associated with the clinical subtype. In contrast to the study by Wolff et al, our group has recently shown that a nonpapillary dermal distribution in children is associated with monomorphic MPCM which is in turn associated with a higher risk of SM.[15] Other histomorphological changes that can be observed in CLM are talengiectatic vessels, papillary dermal edema, eosinophils among the dermal infiltrate, and an increased basal epidermal pigmentation.

In addition to the gross distribution pattern, a more interstitial distribution of MCs (and loss of periadnexal distribution) of MC is found in adult-type mastocytosis versus inflamed controls. Overall, MC number, distribution, and clustering as well as basal pigmentation were proposed as minor criteria in a scoring model for MPCM in adults versus inflamed controls.[6] In the future, digital pathology and the use of artificial intelligence could help the pathologist in the assessment of these histopathological features for a more specific diagnosis of mastocytosis.

IMMUNOPHENOTYPE

The aberrant expression of CD2, CD25, or CD30 on BM MC is important to differentiate SM from CM. However, only few studies analyzed the specificity of these markers in skin. In adults, Lange et al[17] demonstrated a significantly higher number of patients with CD2-positive MC, but not CD25, in a cohort of 52 adult patients presenting with MPCM with SM compared with CM. The strength of this study is that patients underwent BM and therefore formed a homogenous population with CM. Notably, the follow-up time was unclear from the study and it cannot be ruled out that these patients will eventually develop SM. Furthermore, sampling error occurs in BM biopsies, in which MC aggregates can be easily missed. By contrast, Hollman et al[18] showed that adults with SM showed significantly more CD25-positive MC compared with patients they defined as "CM." However, only 5 of 20 patients classified as "CM" had undergone BM examination to exclude SM. According to the current WHO classification, the remaining 16 patients with "CM" would have been classified as MIS and not as CM, not in the least because their follow-up in almost half of their "CM" patients was not available. Probably, most of the 16 patient classified as CM would eventually have been classified as SM. In contrast to the aforementioned studies, others have shown fully negative staining for CD2 and CD25 in adults and children[19] or adults alone[6] with CLM. Berezowska et al[20] identified only a small percentage of CD25-positive MCs in the skin of patients with SM, while most BM MCs were CD25 positive. Our group recently reported no differences in CD2, CD25, and CD30 expression between children and adults with CM and SM.[15]

CD30 is another marker to define neoplastic MCs and was recently evaluated by Russano et al in a retrospective cohort of 42 cases, of which 13 adults fulfilled criteria for SM and 29 for CM of which 11 concerned children.[21] Although a major weakness of this study is the lack of statistical analyses, it was found that CD30 is also highly expressed in children with CM. Furthermore, CD30 intensity was found weaker on MC in SM compared with CM. Whether these findings were statistically significant and whether CD30 expression in adults with SM versus adults with CM was different were not analyzed. Poirier et al[22] analyzed the expression of CD30 in BM versus skin biopsies in adults with different types of SM. In contrast to BM CD30 MC expression, expression of CD30 in skin biopsies was not associated with the clinical course. The only significant finding in skin was the association of CD30 positivity with skin fold distribution and dermal sheet–like pattern on histology, which could potentially have therapeutic but not prognostic consequences. In a study by Greenberger

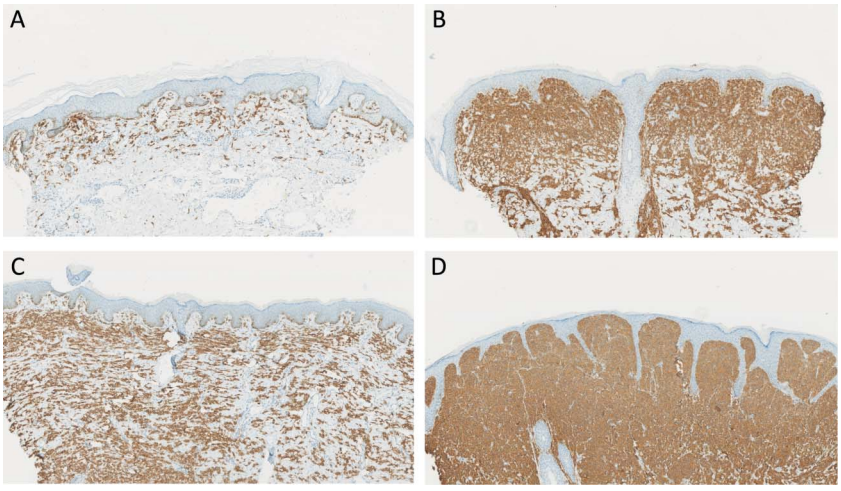

FIGURE 2. Examples of MCs visualized by CD117 immunohistochemistry showing the 4 distribution patterns as recognized by Wolf et al: (A) perivascular in the papillary body and upper dermis, (B) sheet-like within the papillary body and upper reticular dermis, (C) interstitial, and (D) nodular.[16]

et al, 84% of patients with pediatric onset mastocytosis were CD30 positive, 60% CD25, and 44% for CD2.[23] The expression of all markers did not associate with the clinical subtypes of mastocytoma and MPCM, nor with clinical course. None of these patients showed extracutaneous disease although almost all patients showed persistent disease. Major limitations of their study were the lack of description of the efflorescence (monomorphic vs. polymorphic), the short follow-up with a mean of 40 months and therefore most children had not reached adulthood yet, and lack of BM analyses.

In conclusion, CD2, CD25, or CD30 expression on MCs is not associated with clinical course or subtype. Prospective studies are warranted, comparing CLM versus inflammatory and healthy controls, as well as comparing CM versus SM. We agree with the consensus paper from the European Competence Network on Mastocytosis that it can be concluded that in contrast to MC in BM, no specific aberrantly expressed marker of clonal MC in the skin has been described to date.[1] With the caveat that, when one believes in the concept of CM versus SM in adults, CD2 is the most promising marker for localization of SM in the skin as published by Lange et al.

MOLECULAR ANALYSIS

Gebhard et al recently found that 28/32 (87.5%) of their adult cases with MPCM revealed a KITD816V mutation in skin, while the other 4 were not evaluable due to poor sample quality. This is comparable with the study by Berezowska et al,[20] who demonstrated the KITD816V mutation in 75% of their cases of SM, who all had the KITD816V mutation present in the BM MCs. By contrast, in the inflamed control group, 23 of 36 patients (63.9%) did not show any KITD816V mutations, while 13 cases were not evaluable. Moreover, their study showed that KITD816V mutational status and MC density in the upper dermis were the strongest discriminators between CLM and inflamed controls. Thus, a KID816V-positive status shows a 100% sensitivity and specificity compared with inflamed controls. However, the technique is expensive and not readily available in nonacademic hospitals, and therefore, the previously described scoring model would be more suitable in general clinical practice. A detectable KITD816V mutation in skin biopsies of adults was also found by others showing this in 72%. In children, the KITD816V mutation was found in 57%. Additional mutations in the KIT gene (V560G) and internal tandem duplications 502–503dupAY were found in 12% of adults and 8% of children. The V560G seemed to be associated with more advanced disease, while all other mutations were not associated with the clinical phenotype.[24] The KIT gene has been sequenced by few studies before, showing somatic mutations mainly in exon 17 D816V,[25–27] and none of the studies found an association with the clinical phenotype or clinical course.

CONCLUSIONS

The histopathological criteria for CLM remain poorly defined due to heterogeneity of the published literature and the lack of prospective comparative studies. MC count is influenced by differences in detection and counting techniques used. Although MC numbers in CLM can be significantly higher compared with inflamed and healthy controls, in some instances, considerable overlap exists in MC numbers. In such cases, a paired analysis of MCs in lesional versus perilesional skin could be an option. It is suggested that a number of MCs between 75 and 250 MCs/mm^2 are a range in which mastocytosis should be considered and above 250 MC/mm^2, a diagnosis of CLM can be made. A recent study showed a high specificity of $>$95% of a MC count $>$139 MC/mm^2 compared with inflamed controls. In difficult cases, ancillary techniques such as molecular analysis with detection of D816V on FFPE have a high sensitivity and specificity for mastocytosis. At this moment, there is no evidence that immunohistochemistry of CD25, CD2, and/or CD30 has any additional value in the diagnosis, subtyping, or clinical course of mastocytosis.

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