Mastocytosis in children:
Clinicopathological study based on 35 cases

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Summary. Immunohistochemical staining is useful in the diagnosis of bone marrow infiltration in systemic mastocytosis. However, it is not clear if antibody staining may be helpful in the diagnosis of cutaneous mastocytosis (CM). We studied the histological appearance of CM in 35 pediatric patients. Cases were assigned to three basic clinical groups: I - Urticaria pigmentosa (UP, n=29); II - Mastocytomas (n=4); and III - Diffuse Cutaneous Mastocytosis (DCM, n=2). The analysis of clinical information revealed an association between the presence of diarrhea and a higher number of cells/field. Nine doubtful cases, all of them macules, were selected based on the scarcity of mast cells (MC) and the absence or rarity of other inflammatory cells. We compared the number of cells identified in Giemsa and immunohistochemical stains in definite and doubtful cases. The intraclass correlation statistic tested the concordance between each staining method. All 9 dubious cases according to the Giemsa stain had their CM diagnosis confirmed by the immunohistochemistry analysis. The intraclass correlation between Giemsa and c-kit was good (0.7) when the number of MC was high. However, there was no correlation between the mast cells counts in the two different stains in the dubious cases. The immunohistochemistry with c-kit might make CM diagnosis easier, especially in the macular cases, when there is a lower number of MC.

Key words: Cutaneous mastocytosis, Children, Urticaria pigmentosa, Mastocytoma, c-kit antibody

Introduction

Mastocytosis refers to a group of disorders characterized by abnormal proliferation of mast cells (MC) in various tissues and organs (Demis, 1963; Longley et al., 1995). It is a clonal proliferation of mast cells where gain of funtion mutations of the c-kit gene have been reported. Recent genetics findings also indicate different pathogenetic forms of mastocytosis, as adult patients and those with associated haematological disease usually express activating mutations of the receptor c-kit, whereas in most cases of childhood-onset mastocytosis such mutations were not reported (Hartmann and Henz, 2001). The heterogeneity of c-kit mutations may have contributed to difficulties in characterizing genotype-phenotype correlations of the disease (Yanagihori et al., 2005). Cutaneous mastocytosis (CM) is defined as an increase of MC in the skin, with three clinical types present in children: urticaria pigmentosa (UP), mastocytomas and diffuse cutaneous mastocytosis (DCM). UP is the clinical prototype of MC disease (Longley et al., 1995; Katsamba et al., 1999). Childhood-onset UP regresses spontaneously in the majority of cases whereas virtually all adult-onset cases persist and may progress to systemic MC disease (Travis et al., 1985; Tebbe et al., 1998; Torrelo et al., 1998; Kiszewski et al., 2004; Ben-Amitai et al., 2005). The difficulties encountered in the diagnosis of CM are due to the MC heterogeneity. Variations in granulation patterns and nuclear morphology in different organs may simulate other diseases. In addition, degranulation and hypogranulation caused by fixation "sensitivity" may cause difficulties in the diagnosis of mastocytosis (Mikhail and Miller-Miliska, 1964; Marshall et al., 1987; Chin-Yang, 2001). New monoclonal antibodies for specific staining of MC became available in the last two decades and their use has been acknowledged in the bone marrow investigation of systemic mastocytosis patients (Horny et

al., 1998; Chin-Yang, 2001; Hans-Peter and Valent, 2001). The c-kit antibody binds to the c-kit receptor present in the membrane of MC. It can be used in routinely processed paraffin sections for identification of MC (Natkunam and Rouse, 2000; Chin-Yang, 2001). This investigation analyzed histologic patterns of 35 pediatric CM biopsies and the clinical-pathological correlation through a morphometric analysis and available clinical data. Moreover, we analyzed the use of the c-kit antibodies in the diagnosis of CM in the dubious cases.

Material and methods

Skin biopsies from 35 patients (25 males and 10 females) with clinical and/or pathological diagnosis of CM underwent histopathological analysis. The ethics committee of the institution approved the investigation. The information about age of onset of lesions were available in 27 out of 35 cases and the information about signs and symptoms in 16 out of 35 cases. All 35 cases were analyzed by Giemsa staining and immunohistochemistry using the c-kit antibody. The paraffin blocks were retrieved and cut. Biopsies were fixed in 4% formaldehyde for 1-2 days and embedded in paraffin. The histological investigation focused on the distribution of the dermal infiltrate and on the number of positive MC in the immunohistochemical staining in each clinical type of CM. Anti c-kit antibody (Dako corporation) was diluted 1:100 and processed through avidine-biotine-peroxidase system with 3,3' diaminobenzidine as chromogen. Nine samples of normal skin from reduction mammoplasties were used as controls. Regarding to the labeling, for the negative control we applied the same immunohistochemistry protocol but without the c-kit antibody. Bone marrow (with diagnosis of leukemia) slides were used as positive controls, since they have many c-kit positive cells.

We analyzed the periadnexal involvement, presence of blisters and the distribution of cellular infiltrates in the dermis. MC were counted with a computerized Leica-Kwin Colour program. The fields analyzed had 400x magnification in an area of 0.013 mm^2. Five random fields were evaluated in the papillary dermis for each staining (Giemsa and c-kit). The criteria for MC identification with Giemsa was the presence of metachromatic intracytoplasmic granules and for c-kit a maroon color in cell membranes.

Nine cases of clinical diagnosis of UP with macular lesions were classified as doubtful because of the very small number of mast cells in the Giemsa stain (similar to the control group). In addition, there was absence or rarity of other inflammatory cells.

Categorical variables were analyzed by chi-square and continuous variables by Mann-Whitney and Kruskal Wallis tests. The significance level was set at $P<0.05$. The intraclass correlation agreement statistic tested the concordance between the number of cells identified in each staining method in definite and doubtful cases. The coefficient represents concordance, where 1 is perfect agreement and 0 is no agreement at all.

Results

The 35 cases were classified as UP (n=29), Mastocytomas (n=4), and DCM (n=2). The average age of onset of lesions was 12.2 months (SD=33.4 months; range: 1 month to 13 years old). The morphometric analysis based on Giemsa and c-kit are presented in Table 1.

The analysis of perianexal distribution in all cases showed MC around hair follicles and eccrine glands regardless of the CM type. The frequency of clinical lesions found in CM types was heterogeneous. Seventy four percent of UP cases presented macules, 40% had papules, 40% plaques and 11% blisters. Two out of the 4 mastocytoma cases had plaques, 1 presented macule and 1 displayed nodule. Both DCM cases presented diffuse thickness of the skin accompanied by blisters.

Patients with diarrhea presented a higher number of cells/field (Table 2).

All 9 doubtful cases according to the Giemsa stain had their CM diagnosis confirmed by the immuno-histochemistry analysis, with an average of 6.4±3.8 MC per field. The intraclass correlation (ICC) between Giemsa and c-kit was good (0.7) when the number of

Table 1. Mast cell number in clinical presentations of 35 cases of cutaneous mastocytosis (Mast cell/field, Mean ± SD).

	UP n=29	Mastocytoma n=4	DCM n=2
Giemsa	30.7±23	30.3±24	48.7±1.8
c-kit	34.3±26	34.7±28	60.6±2.2

UP: urticaria pigmentosa; DCM: diffuse cutaneous mastocytosis. All comparisons among clinical presentations revealed non-significant differences.

Table 2. Average number of mast cells/ field according to signs and symptoms in mastocytosis cases.

Clinical information (n=16)	Mast cells/field* Mean±SD	P
Diarrhea		
Yes (2)	73.5±15.9	0.03
No (14)	30.4±22.6	
Pruritus		
Yes (8)	46.8±22.9	0.08
No (8)	24.7±25.3	
Hepatomegalia		
Yes (3)	56.6±31.2	0.15
No (13)	30.9±23.4	

*c-kit stain, Mann-Whitney test

MC was high. However, the doubtful cases presented no correlation (0.0) (Table 3). The comparison between c-kit and Giemsa stains in dubious cases is also shown in Figure 1.

The control group presented an average of 1.0±0.7 cells/field in the c-kit stain and 0.9±0.3 cells/field in the Giemsa stain.

Table 3. Average number of mast cells/ field according to diagnostic status in Urticaria Pigmentosa cases (Mast cells/field).

Diagnostic status*	Giemsa Mean±SD	c-kit Mean±SD	Intraclass correlation**
Definite diagnosis UP (n=20)	30.7±23.3	34.3±25.7	0.7
Dubious cases (n=9)	1.3±0.9	6.4±3.9	0.0

*: Diagnosis by histologic examination, based on the quantity of Mast Cells. **: Intraclass correlation between Giemsa and c-kit stains.

Discussion

When there is a small number of MC in the Giemsa stain, the histologic diagnosis of CM is difficult. We verified that Giemsa stain was reliable when the number of MC was high. However, when there was a small number of cells, the c-kit stain revealed a higher number of MC, while the Giemsa stain did not differentiate such cases from the control group. Therefore, the immunohistochemistry with c-kit may help the diagnostic process in doubtful cases.

Giemsa stain usually identifies MC by their metachromatic granules in the cytoplasm (Marshall et al., 1987; Markey et al., 1989). Until recently, a reliable immunohistochemical marker for detection of MC in paraffin-embedded tissue was not available. Metachromatic stains, such as Giemsa and toluidine blue are effective in highlighting cytoplasmatic granules, but degranulated MC may remain undetected (Olafsson et al., 1986). The reactivity of MC was evaluated for a
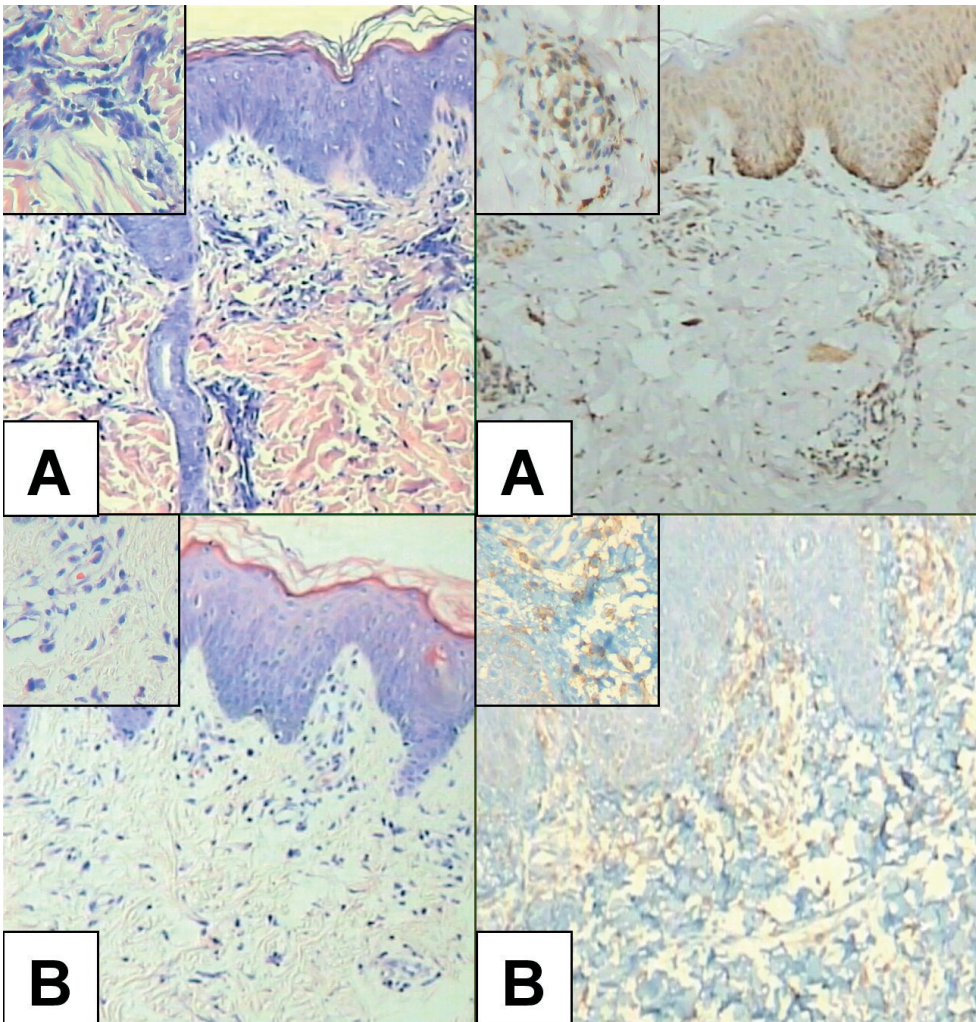

Fig. 1. Giemsa and c-kit stains in dubious cases. **A and B** correspond to two different patients, **left** and **right** are Giemsa and c-kit stains, respectively. Note the large number of cells identified by c-kit stain while Giemsa reveals a small number of metachromatic granules. x 100; inserts, x 400

variety of immunohistochemical markers including CD68, CD2, PG-M1, HAM56, MAC387, LN5, MAX1,3,11 and 24 and anti-tryptase (Horny et al., 1993, 1998; Hamann et al., 1995; Hans-Peter and Valent, 2001). The specificity of these markers, however, includes cells of the myeloid and myelomonocytic derivation. Anti-c-kit (CD117) has been established as a specific and sensitive marker for MC in paraffin sections (Arber et al., 1998; Chin-Yang, 2001; Baek et al., 2002). It is directed against the c-kit membrane receptor. Therefore, anti-c-kit can label MC even if they are degranulated. There are no evidences suggesting lack of c-kit reactivity when the c-kit gene is mutated. It is unlikely that mutations in the c-kit gene could cause lack of reactivity, since the antibody is directed against the extracellular domain (immunoglobulin-like domain) of the receptor, while the c-kit mutations described in mastocytosis alter the transmembranal and intracellular domains (Yanagihori et al., 2005; Dirnhofer et al., 2006). Natkunam showed in blood marrow samples that CD117 was a valuable marker in distinguishing systemic MC disease from the other hematolymphoid lesions exhibiting notable histologic overlap (Natkunam and Rouse, 2000). In the present study, we verified that anti-c-kit antibody stained the highest number of MC in all clinical subtypes of CM and was of particular utility in cases with few MC in the histology.

There is a lack of consistency across studies in the method used to report the number of MC, making comparisons among the studies extremely difficult (Mikhail and Miller-Miliska, 1964; Olafsson et al., 1986; Kasper and Tharp, 1987; Kasper et al., 1987; Garriga et al, 1988). Even in normal skin studies, there is a significant variation in the reported number of MC (from 44 to 108 MCs/mm^2). Janssens et al. (2005) reported significant differences in the number of MC in relation to the site of origin of biopsy, with greater amounts of MC at distal body sites. The average number of MC in normal breast skin in our control group was 77 MC/mm^2, consistent with Janssens et al. findings in the trunk (78.6 MCs/mm^2).

Many authors have tried to correlate the clinical type of CM with distribution of MC seen in the skin without success, or detecting only weak correlations (Mihm et al., 1973; Travis et al., 1998; Wolff et al., 2001). Four patterns of MC infiltrates can be recognized in dermatopathology (Mihm et al., 1973): (A) perivascular and upper dermis; (B) sheet-like with the papillary body and upper reticular dermis; (C) interstitial and (D) nodular. In each clinical subtype of CM there is a variety of clinical lesions (Stein, 1986; Horan et al., 1992). There seems to exist a better correlation between the patterns of MC infiltrate and the number of MC with macroscopic appearance of a particular lesion rather than with the clinical type of CM (Kasper and Tharp, 1987; Kasper et al., 1987). As far as we know, there are no reports on the number of MC in DCM. In the present study, when compared to controls, c-kit staining revealed nearly 60 times more MC in DCM. There are

descriptions of tumoral infiltrates affecting the papillary dermis and extending into the subcutaneous tissue in nodular lesions of UP (Mihm et al.,1973). It is well known that nodular lesions yield the highest number of MC (up to 185 times compared to normal skin) when they exhibit a lichenoid (sheet) infiltrate or nodular (tumoral) aggregates (Kasper and Tharp, 1987; Kasper et al., 1987; Garriga et al., 1988). The perivascular pattern is found in small, flat, macular forms of UP that on the other hand contain the lowest increase in the number of MC (4-16 times higher than in normal skin) (Mikhail and Miller-Miliska, 1964; Kasper et al., 1987; Garriga et al., 1988; Wolff et al., 2001). When the number of MC within lesions is low, it sometimes overlaps with the number of MC in normal skin (Janssens et al., 2005). There exists no known cut-off point for a decision whether a MC count is within the limits of normal skin or increased. We established the cut-off for definite cases in 5 times more MC than controls based on previous reports (Mihm et al., 1973; Wolff et al., 2001). Doubtful cases were thus analyzed separately. In the doubtful cases reported here (corresponding to macular UP lesions) the c-kit staining revealed an average observed number of MC at least 5 times that found in the control group, confirming the diagnosis of CM.

Some studies have tried to correlate histological findings from skin biopsies with patient prognosis. At the moment, examination of skin biopsies permits only a diagnosis of CM and does not help to predict the risk of systemic involvement (Travis et al., 1998).

The present study showed a significant association between the number of MC and the presence of diarrhea, and a trend for pruritus and hepatomegaly. The type of CM that presents more frequently signs and symptoms was DCM, confirming previous studies (Torrello-Fernandez et al., 1998; Kiszewski et al., 2004). However, a larger sample size is needed for a definitive conclusion.

The immunohistochemistry with c-kit might make diagnosis easier, especially in the macular cases, when there is a lower number of MC. On the other hand, Giemsa stain presented a good ICC with c-kit in cases with numerous MC, and may be preferred because it is cheap and easy to perform, and reliable in most cases. The absence of MC in metachromatic staining in cases with a clinical diagnosis of CM should lead the pathologist to perform an immunohistochemistry study before the conclusion of the diagnosis. A positive staining of c-kit may avoid a new skin biopsy, a sometimes difficult procedure in childhood.

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