Cutaneous mastocytosis, problems of clinical diagnosis of four cases

VIRGIL PĂTRAȘCU1, ANDREEA-OANA ENACHE1, RALUCA NICULINA CIUREA2, CORNELIU CRISTIAN GEORGESCU3, ALINA MARIA VILCEA1, LOREDANA ELENA STOICA1, MARIUS EUGEN CIUREA1

1Department of Dermatology, University of Medicine and Pharmacy of Craiova, Romania
2Department of Pathology, University of Medicine and Pharmacy of Craiova, Romania
3Clinic of Anesthesia and Intensive Therapy, Emergency County Hospital, Craiova, Romania
4Department of Plastic Surgery, University of Medicine and Pharmacy of Craiova, Romania

Abstract
Mastocytosis is a rare disease characterized by a pathological increased of mast cells in one or more tissues, particularly in the skin, bone marrow, liver, spleen, lymph nodes and gastrointestinal tract. Cutaneous mastocytosis represents over 90% of cases found with predilection in children. The aim of the paper was to summarize the authors’ clinical, histopathological and immunohistochemical observations on patients with cutaneous mastocytosis. We present four cases of cutaneous mastocytosis, sporadic form, customized by clinical presentation and age of onset: two installed in the neonatal period, a case with onset in infancy and another in adulthood. For the assessment of the severity and the effectiveness of the treatment, we used SCORMA index. We performed in each patient histopathological examination of the skin (Hematoxylin–Eosin and Giemsa stains), the dosage of mediators (serum tryptase level, serum histamine levels, urinary histamine metabolites) and the balance expansion (complete blood cell count, liver biological investigations, abdominal ultrasound, skeletal radiography, chest radiography).

For the adult with mastocytosis, we performed abdominal scanner and cytological study of the bone marrow. Following investigations carried out in each case, we mentioned the diagnosis of cutaneous mastocytosis, and also excluded several diseases confounded by clinically and histologically aspect. Considering the fact that the balance expansion was negative, we excluded the diagnosis of systemic mastocytosis. The presence of anemia and protein energetic malnutrition in children with mastocytosis involves carrying out balance extension for the exclusion of a systemic form of the disease. Histopathological examination of the skin using special stains, the dosage of mediators (serum tryptase level, serum histamine levels, urinary histamine metabolites) and the balance expansion establish the diagnosis of cutaneous mastocytosis and also exclude many confusions because of the clinical presentation.

Keywords: mastocytosis, cutaneous mastocytosis, children, mast cell, histopathology.

Introduction
Mastocytosis is a rare disease characterized by a pathological increased of mast cells in one or more tissues, particularly in the skin, bone marrow, liver, spleen, lymph nodes and gastrointestinal tract [1]. Cutaneous mastocytosis represents over 90% of cases found with predilection in children [2].

Mastocytosis was first described by Nettleship and Tay as a “Rare form of Urticaria” in 1869, and in 1936, Sezary et al. used the term mastocytosis to describe patients with cutaneous and systemic affection [3–5].

There are two general forms of mastocytosis: cutaneous (increase of mast cells in the skin) and systemic (mast cell infiltration of the bone marrow, gastrointestinal tract, liver, spleen, and lymph nodes). According to the latest World Health Organization (WHO) classification, there are seven variants of mastocytosis: cutaneous mastocytosis, indolent systemic mastocytosis, systemic mastocytosis with associated clonal hematological non-mast cell lineage disease, mast cell leukemia, extracutaneous mastocytosis, mast cell sarcoma and aggressive systemic mastocytosis [6].

Cutaneous mastocytosis (CM) represents the vast majority of the mastocytosis. The estimated prevalence of mastocytosis in Central Europe is 0.005–0.01% [7].

The aim of the paper was to summarize the authors’ clinical, histopathological and immunohistochemical observations on patients with cutaneous mastocytosis.

Patients and Methods
We present four cases of cutaneous mastocytosis, sporadic form, customized by clinical presentation and age of onset: two installed in the neonatal period, a case with onset in infancy, and another in adulthood. Patients had been hospitalized within the Clinic of Dermatology, Emergency Hospital, Craiova, Romania, between 2012 and 2013. The age of the patients was between 10 months and 28 years.

We performed skin biopsies from all the four patients (three children and one adult) with clinical features of cutaneous mastocytosis. All the cases were analyzed by classical Hematoxylin–Eosin (HE) and Giemsa stainings, and immunohistochemistry using the CD117 (c-kit) antibody (clone T595). Biopsies were fixed in 4% formalin for 1–2 days and embedded in paraffin. The paraffin blocks were retrieved and cut. For immunohistochemistry, we used CD117 (c-kit) antibody (Novolink, Leica Corp.), diluted 1:40, treated with citrate and incubated for one hour at 25°C with the primer. The histological investi-
igation was focused on the distribution of the dermal infiltrate. We analyzed the periannexal and perivascular involvement and the distribution of mast cells infiltrates in the dermis.

We performed in each patient the dosage of mediators (serum tryptase level, serum histamine levels, and urinary histamine metabolites) and the balance of expansion, which was negative.

Balance of extension meant for each patient complete blood cell count, liver biological investigations, abdominal ultrasound, skeletal radiography, chest radiography. For the adult with mastocytosis, we performed abdominal scanner. For the assessment of the severity and the effectiveness of the treatment, we used SCORMA Index [3].

**Results**

**Case No. 1**

A 1.5-year-old patient was consulted for a disseminated skin eruption, well defined, brownish plaques with variable dimensions, between 2 cm and 5 cm (Figure 1, a and b). The first lesions occurred at the age of three weeks. Within six weeks have developed the other lesions, later the evolution was stationary.

Based on clinical examination, histopathological and immunohistochemical findings (Figure 1, c–e) and also paraclinical aspects, our diagnosis was cutaneous mastocytosis, plaque form with the onset in the neonatal period; protein energy malnutrition II. The SCORMA Index was 47.8.

**Case No. 2**

A 10-month-old patient was hospitalized for multiple brown macules, ranging in size from 0.3 cm to 1 cm, well defined, disseminated (Figure 2, a and b). The lesions emerged gradually from six months. Darier’s sign was present. Histopathological and immunohistochemical findings (Figure 2, c–e) confirmed the diagnosis urticaria pigmentosa and SCORMA index was 21.8.

**Case No. 3**

A 1.8-year-old patient showed yellowish papules, smooth, elastic consistency, some with a small halo of hyperpigmentation, others with transitory perilesional erythema, which mainly interested the trunk (Figure 3, a and b). The first papules appeared on the posterior thorax during the second week of life, their number increasing in the last year. Following clinical examination, histopathological and immunohistochemical findings (Figure 3, c–e) and laboratory explorations, we established the following diagnoses: xanthelasmoidal mastocytosis with onset in neonatal period; deficiency anemia, hypoproteinemia, hypocalcemia. The SCORMA index was 34.4.
Figure 2 – (a and b) Multiple brown macules, ranging in size from 0.3 to 1 cm, well defined, disseminated; (c) Small amount of mast cells in the reticular dermis, HE staining, ×100; (d) Small amount of mast cells in the reticular dermis, Giemsa staining, ×100; (e) Mast cells moderately expressing CD117 antigen, small amount of mast cells, ×100.

Figure 3 – (a and b) Yellowish, smooth papules, elastic consistency, with a small halo of hyperpigmentation or with perilesional erythema; (c) Clusters of mast cells in the papillary dermis extending into the reticular dermis, with perivascular and periaxial distribution, HE staining, ×100; (d) Abundant mast cell infiltrate occupy the entire papillary body and upper reticular dermis, having citoplasmatic granulations, Giemsa staining, ×400; (e) Mast cells strongly expressing CD117 antigen, ×200.
Case No. 4

A 28-year-old patient presents with a maculopapular eruption, consisting of brown lesions, 3–10 mm diameter, on the trunk, neck and thigh region (Figure 4, a and b). The disease started five years ago and was accentuated in the last 3–4 months. Darier’s sign is present. Following histopathological and immunohistochemical findings (Figure 4, c–e), determination of environmental and extension balance, we diagnosed cutaneous mastocytosis, as maculopapular form, with adult onset. The SCORMA index was 43.2.

Clinical examination, the dosage of mediators, histopathological and immunohistochemical examination allowed us to exclude other diagnoses.

In the first case, we have done the clinical differential diagnosis with manifestations of atopic dermatitis; nummular eczema; urticaria; ring angiomas; post-inflammatory hyperpigmentation; cutaneous drug reactions.

We made the differential diagnosis of urticaria pigmentosa with: postlesional hyperpigmentation, “café-au-lait” spots, patches of neurofibromatosis, melanocytic nevi, Albright syndrome, Leopard syndrome, macular and pigmentary amyloidosis.

In the case of xanthelasmoidal mastocytosis, we excluded diseases such as: juvenile xanthogranuloma, eruptive xanthomas, Langerhans cell histiocytosis, eruptive dermatofibromas, pseudolymphoma, leukemia cutis.

In Case No. 4, the differential diagnosis was made with: lichen planus, papular mucinosis, macular and pigmentary amyloidosis, secondary syphilis, cutaneous drug reactions.

In all four patients, we were able to control mastocytosis flare with mast cell stabilizers (Ketotifenum), moderate topical steroids (1% Methylprednisolonum aceponat) and avoidance of triggering factors of mast cells degranulation.

Figure 4 – (a and b) Maculopapular eruption, consisting in brown lesions on the trunk, neck and thigh region; (c) Mast cell infiltrates are seen in perivascular distribution mainly in the papillary body and upper reticular dermis but can also be detected following a few larger vessels in the deep reticular dermis, HE staining, ×40; (d) Mast cell infiltrates are seen in perivascular distribution, Giemsa staining, ×400; (e) Few mast cells, moderately expressing CD117 antigen, ×200.

Discussion

Mastocytosis occurs equally in both sexes, and is more common among the Caucasian population. Although the occurrence of mastocytosis is usually sporadic, there have been approximately 70 familial cases of mastocytosis reported, including at least 15 sets of monozygotic twins [8]. The four cases we presented were sporadic forms of mastocytosis.

Familial mastocytosis has been reported in three generations of one family [9]. Cutaneous mastocytosis occurs most often in childhood, onset period observed by us in three of the four patients. We underline the fact that two of our cases of mastocytosis started in the neonatal period. The age of mastocytosis onset is very important because it has prognostic involvement [10].

In Spain, a prevalence of 5.4 cases/1000 pediatric patients was found, whereas in Mexico the condition was seen in 1:500 pediatric patients.

The etiopathogeny of mastocytosis is not fully understood [11]. They reveal changes in the structure and the activity of kit receptor expressed on mast cells (MC), melanocytes, and other cells. Stem cell factor (SCF) is
the ligand for the transmembrane tyrosine kinase receptor c-kit, and is an important growth factor for the final maturation and development of mast cells.

Mutations of these proto-oncogenes have been described in mastocytosis. Based on these observations, mastocytosis is considered a clonal disease rather than a reactive one.

Uncontrolled proliferation of mast cells in this disease seems to be secondary to abnormalities of ligand–receptor (c-kit ligand).

Recently, the immunohistochemical studies in subjects with mastocytosis identified both in the skin lesions as in healthy skin; free SCF growth factor in the extracellular space between the dermis and keratinocytes suggests the existence of a soluble form of this protein. Local accumulation of soluble SCF in the skin of patients with mastocytosis is probably responsible for cutaneous pigmentation observed in cutaneous mastocytosis, by stimulating the melanocytes kit [12].

When there are a small number of mast cells in Giemsa staining, histopathological diagnosis of MC is difficult. It was verified that Giemsa reliable when the number of mast cells was high. However, when there has been a small number of cells, the marker CD117 (c-kit) revealed a greater number of mast cells, while Giemsa staining did not differentiate such cases in the control group. Therefore, immunohistochemistry with c-kit may help diagnosis of certainty [13].

Giemsa staining usually identify mast cells through their metachromatic granulations in the cytoplasm [14, 15].

Until recently, there was not available a special marker for immunohistochemistry.

Metachromatic stains such as Giemsa and Toluidine Blue are effective in highlighting cytoplasmic granulations, but cannot identify degranulated mast cells. Mast cell reactivity to a variety was evaluated by immunohistochemical markers including CD68, CD2, PG-M1, HAM56, MAC387, LN5, MAX1, 3, 11 and 24 and the anti-tryptase [16, 17]. However, the specificity of these markers, include the myeloid cells and myelomonocytic derivatives.

Anti-c-kit (CD117) marker was established as a specific and sensitive for identifying of mast cells in paraffin sections [18, 19]. It is directed against membrane receptor c-kit. Therefore, the anti-c-kit may be labeled even degranulated mast cells. There is no evidence suggesting lack of reactivity of c-kit when it is mutated. It is likely that mutations of the c-kit gene can lead to a lack of responsiveness, because the antibody is directed against the extracellular domain (immunoglobulin domain) of the receptor, while the c-kit mutations in mastocytosis described altering the transmembrane and intracellular [20, 21].

CD117 has been a valuable marker of mastocytosis differentiation from other lesions, which looks histopathological hematolymphoid overlapping [22]. Some studies have shown that CD117 scored the highest number of mast cells in all clinical subtypes of cutaneous mastocytosis and was particularly useful in cases with few mast cells identified by histopathology classic. Even studies on normal skin, there is a significant variation in the reported number of MC (44–108 MCs/mm²). Immunohistochemical examination of CD117 could make diagnosis easier, especially in cases where there is a smaller number of MC. On the other hand, Giemsa may be preferred in cases with numerous MC accumulation, as it is inexpensive and easy to perform and reliable in most cases.

MC absence in metachromatic staining in cases with a clinical diagnosis of CM should lead pathologist to perform immunohistochemistry with CD117 for mastocytosis certification.

A positive marker CD117 can avoid further skin biopsy, a procedure difficult especially in children.

Activating mutations of c-kit at codon 816 (Asp816) are associated with constitutive activation of tyrosine kinase and promote ligand-independent autophosphorylation of the receptor, giving a survival advantage to activated mast cells compared to normal cells.

D816V mutation is found in 80% of patients with systemic mastocytosis (SM) and may represent a potential therapeutic target [23].

Recently, were identified mutations that may play a role in the etiology of mastocytosis in some patients, such as V560G (juxtamembranar in the kit) detected in mast cell leukemia, D816Y, D816F, D816H, and E839K (inactivating mutations) in some reported cases of mastocytosis in children.

Extremely rare mutations present in <1% of patients with mastocytosis, includes R815K, D820G, V533D, V559A, del419, K509I and A533D4 [24].

The c-kit activation by stem cell factor is one of the mechanisms proposed to explain the pathogenesis of neuroblastoma, small cell lung cancer and colon and breast cancer [25].

The most common clinical types in children are urticaria pigmentosa (47–75% of cases) and mastocytoma (17–51% of the cases) [26]. Referring to our patients, two of them showed rare forms of mastocytosis: plaque form of cutaneous mastocytosis and the other case with xanthelasmoidal mastocytosis. The most common localizations of mastocytosis have been the trunk and the limbs, such data from the literature [27].

Mast cell degranulation and secondary release of preformed mediators (histamine, heparin, tryptase, chymase), newly generated (leukotrienes, prostaglandins, cytokines, SCF, TGF-β, TNF-α, IL-5, IL-6, IL-16) is responsible for clinical manifestations such as urticaria, flushing, pruritus, bronchoconstriction, nausea, vomiting, diarrhea, malabsorption, tachycardia, hypotension, syncope, purpura, bleeding, cachexia, osteoporosis, fibrosis, fever, etc. [28, 29]. These manifestations are inconstant found in cutaneous mastocytosis.

We held the cutaneous mastocytosis diagnosis based on histopathological findings (HE and Giemsa stainings), the dosage of mediators (serum tryptase level, serum histamine levels, urinary histamine metabolites) and the balance of expansion, which was negative [30].

One broad spectrum of neoplastic and reactive conditions has to be included in the differential diagnosis of MS:

- Mast cell hyperplasia is completely benign and characterized by an increase in loosely distributed, round, mature mast cells, which do not form compact infiltrates, without immunophenotypical aberrations and mutations of c-kit.
Myelomastocytic leukemia is an extremely rare myeloid neoplasm with marked signs of differentiation of mast cells line, but diagnostic criteria for systemic mastocytosis are not met. Cytomorphological metachromatic blast cells containing some granulations are essential finding to establish the diagnosis.

Myeloid/lymphoid neoplasms with eosinophilia may contain a large number of atypical, spindle shaped mast cells, CD25+ with a tendency to form small groups, but the compact infiltrates, characteristic for mastocytosis, are almost never seen. So far, KIT-D816V has not been detected in these tumors [31].

In pediatric patients, the bone marrow biopsy is indicated only if the signs of systemic disease or increased levels of serum tryptase (>100 ng/mL) are present [32].

At present, there is no curative treatment for cutaneous mastocytosis. Cutaneous mastocytosis treatment is only symptomatic. It may include medications such as: potent topical steroids, mast cell stabilizers, leukotriene antagonists. We succeed to control mastocytosis flare with mast cell stabilizers, moderate topical steroids and avoidance of triggering factors of mast cells degranulation.

H2-antihistamines and proton pump inhibitors are used to treat gastric hypersecretion associated with histamine release in mastocytosis.

Disodium Cromoglycate has good effect on digestive manifestations. In severe forms can be used corticosteroids and PUVA therapy.

PUVA therapy has been shown to be highly effective on congestive skin manifestations, but it needs a period of about 4–10 weeks [33].

Imatinib Mesylate (STI-571, Gleevec) is a selective tyrosine kinase (210 protein) inhibitor produced by the chimeric BCR-ABL gene located on chromosome 22 and represents the first line of treatment for patients with chronic myeloid leukemia and also inhibits the c-kit receptor [34].

Imatinib has been used successfully in treating SM but its role in cutaneous mastocytosis treatment is unclear.

A recent study in children with cutaneous mastocytosis showed c-kit mutations comparable to those of the SM patients [35].

Regardless of the clinical form is recommended a lifestyle with exclusion of the factors that stimulate mast cell degranulation.

In a study published in 2011, the authors concluded that mastocytosis affects the quality of life in patients with onset of disease in childhood, with a high degree of social and recreational activities limitation and depression was found in 56% of pediatric patients.

This high prevalence of depression among patients with mastocytosis suggests an impairment of brain probably by mast cell mediators such as serotonin, substance P and cytokines. Recent studies have suggested that the mast cells are involved in the mechanisms for regulate emotions [36–38].

We recommended to our patients microclimate of psycho emotional protection.

In all four patients, we were able to control mastocytosis flare with mast cell stabilizers, moderate topical steroids and avoidance of triggering factors of mast cells degranulation.

As for the evolution and prognosis of these diseases, the literature data show that over 50% of children with cutaneous mastocytosis heal until adolescence, which makes us optimistic on the three pediatric cases.

In children with involvement also in adolescence, the disease course is similar to adult onset mastocytosis (15–30% of cases with systemic involvement).

Association of urticaria pigmentosa with malignancy has been reported but is rare in children.

Murphy et al. reported a case of cutaneous mastocytosis with fatal outcome [39].

All patients will be examined quarterly (dermatological, biochemical). Given that systemic mastocytosis are 25% of adult mastocytosis, the fourth patient will be closely monitored to surprise a possible visceral involvement [40].

Conclusions

The presence of anemia and protein energetic malnutrition in children with mastocytosis involves carrying out balance extension for the exclusion of a systemic form of the disease. Histopathological examination using special stains, the dosage of mediators (serum tryptase level, serum histamine levels, urinary histamine metabolites) and balance expansion establish the diagnosis of cutaneous mastocytosis and also exclude many confusions because of the clinical presentation.

Author contribution

All authors have equally contributed to the realization of the article.

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Corresponding author
Virgil Pătrașcu, Associate Professor, MD, PhD, Department of Dermatology, Faculty of Medicine, University of Medicine and Pharmacy of Craiova, 2 Petru Rareș Street, 200349 Craiova, Romania; Phone +40724–273 676, e-mail: vm.patrascu@gmail.com

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