CASE REPORT

Histiocytic Sarcoma with Visceral Spread Resembling Metastasizing Poorly Differentiated Mast Cell Tumor in a 13 Year Old Dog

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ABSTRACT

We describe an unusual case of disseminated histiocytic sarcoma (DHS) in a 13-year-old spayed female mixed breed Cocker. It started as a periocular multifocal nodular dermatopathy, without any other clinical and hematological abnormalities. In the process of clarifying the diagnosis, first test performed was cytologic examination of the fine needle aspirate, which was highly suggestive of a cutaneous poorly differentiated multifocal mast cell tumor (MCT). Deteriorating health status led to the decision for euthanasia, followed by necropsy. Based on histopathology and immunohistochemical stain, a final diagnosis of disseminated histiocytic sarcoma was made.

INTRODUCTION

The category of discrete round cell neoplasms is composed primarily of cells of the hemolymphatic system. The distinguishing morphologic feature of the round cell neoplasms is the discrete nature of the neoplastic processes. Because there are no cellular junctions connecting individual neoplastic cells as with epithelial neoplasms, this becomes a highly distinguishing feature (DeNicola, 2008). Included in this group are mast cell tumor, histiocytic proliferative disorders (HPD), lymphoproliferative diseases, plasmacytoma, transmissible venereal tumor, melanocytic tumors and undifferentiated round cell tumors (Duncan & Prasse, 1979; Gross et al., 2005, Langova, 2007; DeNicola, 2008).

Diagnosis of canine cutaneous round cell tumors based on cytology and histology, using routine stains, is often challenging. Different round cell tumors may have a similar morphologic appearance, particularly for poorly differentiated tumors. An accurate diagnosis is important in determining prognosis and treatment. Immunohisto-chemistry (IHC) has been used as an adjunct to light

microscopy in the diagnosis of various neoplasms, including round cell tumors (Fernandez et al., 2005).

Histiocytic proliferative disorders belong to the confusing, controversial and poorly defined tumors in dogs (as well as in humans). HPDs are a frustrating group of diseases not only because the diagnosis is often difficult, but also because the clinical presentation, behavior and responsiveness to treatment vary tremendously. The etiopathology and exact pathogenesis of HPD remain largely unknown (Langova, 2007; Ginn et al., 2007). The benign or reactive canine histiocytic proliferative diseases are cutaneous histiocytoma, cutaneous histiocytosis (CH) and systemic histiocytosis (SH). The malignant forms of the histiocytic proliferative disorders are localized and disseminated histiocytic sarcoma (LHS and DHS)(previously called malignant histiocytosis) and haemophagocytic histiocytic sarcoma (HHS) (Ginn et al., 2007; Jacobs, 2002; Moore et al., 2006; Soare et al., 2012).

Disseminated histiocytic sarcoma is a rapidly progressive disease seen in middle-aged to old dogs. Bernese mountain dogs, rottweiler, golden and Labrador

retrievers, and flat-coated retrievers of either sex are most often affected. The condition has been reported in the spleen, lung, liver, lymph nodes, bone marrow, central nervous system, kidneys, skeletal muscle, stomach, vertebral bodies, and adrenal glands. Cutaneous involvement is rare. The age at presentation ranges from 3-11 years and there is no sex predilection. The prognosis is usually extremely poor, the condition is rapidly progressive and there is no known successful therapy (Moore, 1984; Moore & Rosin, 1986; Affolter & Moore, 2002).

Histiocytic sarcomas primarily composed of round cells may mimic morphologically the grade III MCT. The difficulty in distinguishing these two neoplasms increases furthermore with poorly differentiated mast cell tumors (Gross *et al*., 2005; DeNicola, 2008). The majority of histiocytic tumors, however, have at least a small subpopulation of tumor cells that are spindled (Gross *et al*., 2005).

The aim of this study is to describe the clinicopathologic features of a disseminated histiocytic sarcoma in a female of mixed-breed Cocker. In this particular case, the condition debuted in the skin of the periocular region. There are few reports of histiocytic sarcoma with the skin as primary location, and in all, the affected regions are adjacent to appendicular joints or the extremities. Furthermore, in this case, the cytological examination led to the erroneous preliminary diagnosis of a poorly differentiated MCT, contradicting the well documented high relevance of cytology in the diagnosis of round cell tumors.

MATERIALS AND METHODS

Case history

A 13-year-old spayed female mixed breed Cocker was presented to a private small animal clinic with multifocal nodular dermatopathy in the periocular region. Complete blood count and abdominal ultrasonography were unremarkable at the time of first presentation. The clinician suspicioned dermatitis and a treatment with antibiotics (amoxicillin and clavulanic acid, 15 mg/kg/bid, PO) and systemic corticosteroids (1mg/kg/d PO) was initiated. This treatment was repeated intermittently over a year's time period. During this time, additional 0.2 to 1.5 cm in diameter, off-white, firm, alopecic, freely moveable over the underlying tissues and pruriginous nodules developed throughout the body. A 10 cm tumefaction of the left cheek, extensive ulceration of the left oral muco-cutaneous junction and purulent conjunctivitis of the left eye would also develop in the final stages of the disease. The fine needle aspirate, taken one year after the initial presentation, was highly suggestive of a cutaneous poorly differentiated multifocal MCT. At this time, the dog had become lethargic and anorexic. Deteriorating health status led to the decision for euthanasia, followed by necropsy.

Cytology of cutaneous neoplasms

The samples for cytological examination were collected by fine needle aspiration (FNA) of cutaneous nodules. Smears were air-dried and stained with May-Grünwald-Giemsa stain (MGG).

Necropsy

Necropsy was performed within 1 hour from the death of the dog and samples were collected from all the examined organs for histopathological investigations.

Histopathology

All collected tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, cut at 3-5 μm, and stained with hematoxylin and eosin (HE). Staining with toluidine blue was performed for cutaneous nodules.

Evaluation of the mitotic index (MI)

MI, defined as the number of mitotic figures per 10 high-power fields, using a 40x objective, was recorded in hematoxylin & eosin stained sections.

Immunohistochemistry

A cutaneous nodule in DHS was subjected for immunohistochemistry staining with CD3 for excluding the possibility of a lymphoma. The histological samples were fixed in 10% neutral buffered formalin. Sections were cut to a thickness of 3μm, on charged slides, deparaffinized in 100% xylene (3 x 2 minutes) and rehydrated through various grades of ethanol (3x 2 minutes) and rinsed with distilled water. Sections were then subjected to heat antigen retrieval by microwaving in preheated citrate buffer pH 9.0 for 20 minutes. Sections were allowed to cool for 20 minutes and rinsed with TBS. Sections were treated for 5 minutes with 3% (vol/vol) H_2O_2 in distilled water to inhibit endogenous peroxidase activity, followed by rinsing in distilled water for 5 minutes. Sections were then incubated with primary antibody for one hour (CD3 1:300). Antibody used was the polyclonal rabbit anti-human T cell CD3 (DAKO, Glostrup, Denmark). A streptavidin-immunoperoxidase staining procedure (Dako) was used for immunolabeling, at room temperature. After washing, the immunoreaction was observed with 3, 3-diaminobenzidine substrate (Dako). Sections were counterstained with Mayer's hematoxylin (Sigma), dehydrated, cleared in xylene, and mounted on cover slips. Positive immunohistochemical control consisted of canine lymph node. Labeling was considered negative or positive according to wether there was cytoplasmic expression of the target molecule.

RESULTS

Cytology of cutaneous neoplasms

The smears contained a small number of round cells with distinct borders, evident anisocytosis, anisokaryosis and variation in nuclear–to-cytoplasmic ratios. The nuclei had clumped chromatin and multiple or irregularly shaped nucleoli (Fig. 5). The pale blue, sometimes vacuolated cytoplasm varied in mount from moderate to abundant. Bi- or trinucleated cells and abnormal mitotic figures were also noted. Mitotic figures were also seen. The smears had various numbers of eosinophils, lymphocytes and neutrophils. Based on the morphology of the cells and knowing that highly undifferentiated MCT have a significantly decreased number of granules (Vail, 1995), or they can even be absent (Zemke *et al*., 2001), a preliminary diagnosis of poorly differentiated MCT was made.
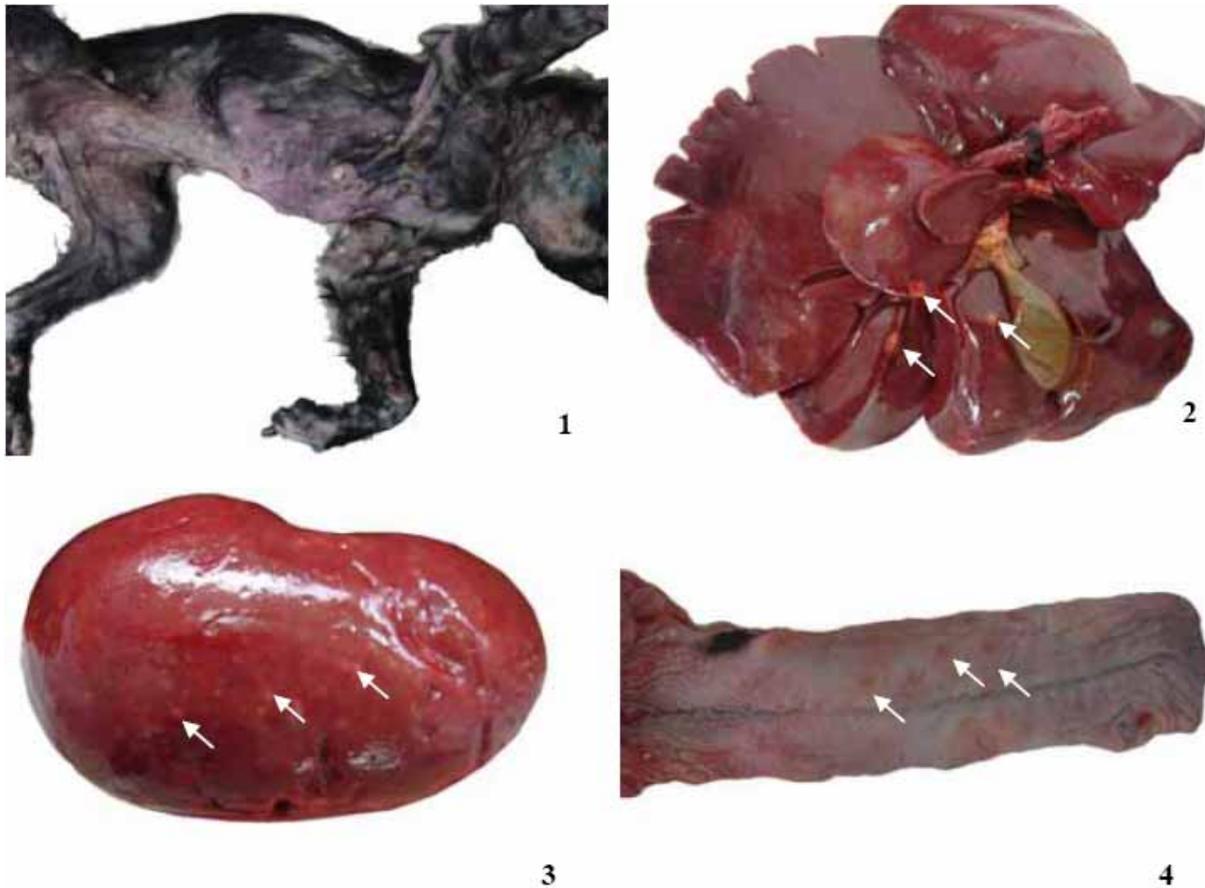
Macroscopic lesions in DHS**. Fig. 1: Skin.** Cutaneous nodules located mainly in the ventral abdominal and thoracic regions (off-white, alopecic, ulcerated or covered with crusts); **Fig. 2: Liver (visceral surface).** Diffuse off-white areas of various dimensions (arrows); **Fig. 3: Kidney.** Multiple small white areas on the surface of the kidney (arrows); **Fig. 4: Tongue (dorsal surface).** Diffuse plaques of various sizes, with central area of erosion (arrows).

Necropsy

Gross examination during necropsy revealed the presence of multiple skin nodules 0.2–5 cm in diameter located mainly on the head, ventral thorax and abdomen (Fig. 1). Some of the nodules would become ulcerated. Multiple, small, white areas up to 1.5 cm in diameter were present on liver (Fig. 2), kidneys (Fig. 3) and tongue (Fig. 4). All the lymph nodes were white and enlarged. Other lesions included hepatomegaly and severe deep pyoderma of ventral neck region, with accumulation of a large quantity of pus. No gross lesions were observed on examination of the brain, skeleton and any other examined organ.

Histology findings

Histologically, the cutaneous tumors (Fig. 6) were found to consist of a mixture of pleomorphic, anaplastic, plump, round to oval and pleomorphic malignant histiocytic cells and spindle-shaped cells. Tumor cells had abundant eosinophilic cytoplasm and occasional cytoplasmic vacuolation. Marked anisocytosis and anisokaryosis were observed. Binucleated or multinucleated neoplastic cells were rare, having nuclei of various sizes and prominent large nucleoli. Mitotic activity was relatively low. Large numbers of eosinophils, lymphocytes and plasma cells were admixed with tumor cells. Multifocal necrosis was also noted. The metastatic

tumors in internal organs had the same histological characteristics as the primary cutaneous tumors. Toluidine blue staining evidenced no metachromatic granules in the cytoplasm of tumor cells, thus invalidating the previous suspicion of poorly differentiated MCT (Fig. 7). The general pattern of the tumor, areas of well differentiated tumor cells and negative results of toluidine blue staining led to the histological diagnosis of disseminated histiocytic sarcoma.

Metastizing neoplastic histiocytes were found in lymph nodes, liver, kidneys, spleen, bone marrow and tongue (Table 1).

Lymph node involvement ranged from small aggregates of neoplastic histiocytes located mainly in the subcapsular sinus, to effacement of normal histologic architecture by tumor cells (Fig. 8). Neoplastic histiocytes had morphological features identical to those described in the skin neoplasms.

The malignant histiocytes were present in the *liver* as multiple nodular aggregates replacing the hepatic parenchyma or distending the sinusoids and portal areas. Large numbers of eosinophils were present in the tumors located in the parenchyma (Fig. 9). Clusters of hematopoietic cells, demonstrating a mild extramedullary hematopoesis and extended areas of necrosis were seen.

The *kidneys* had multifocal infiltration with tumor cells and plasma cells in the interstitial tissue and in
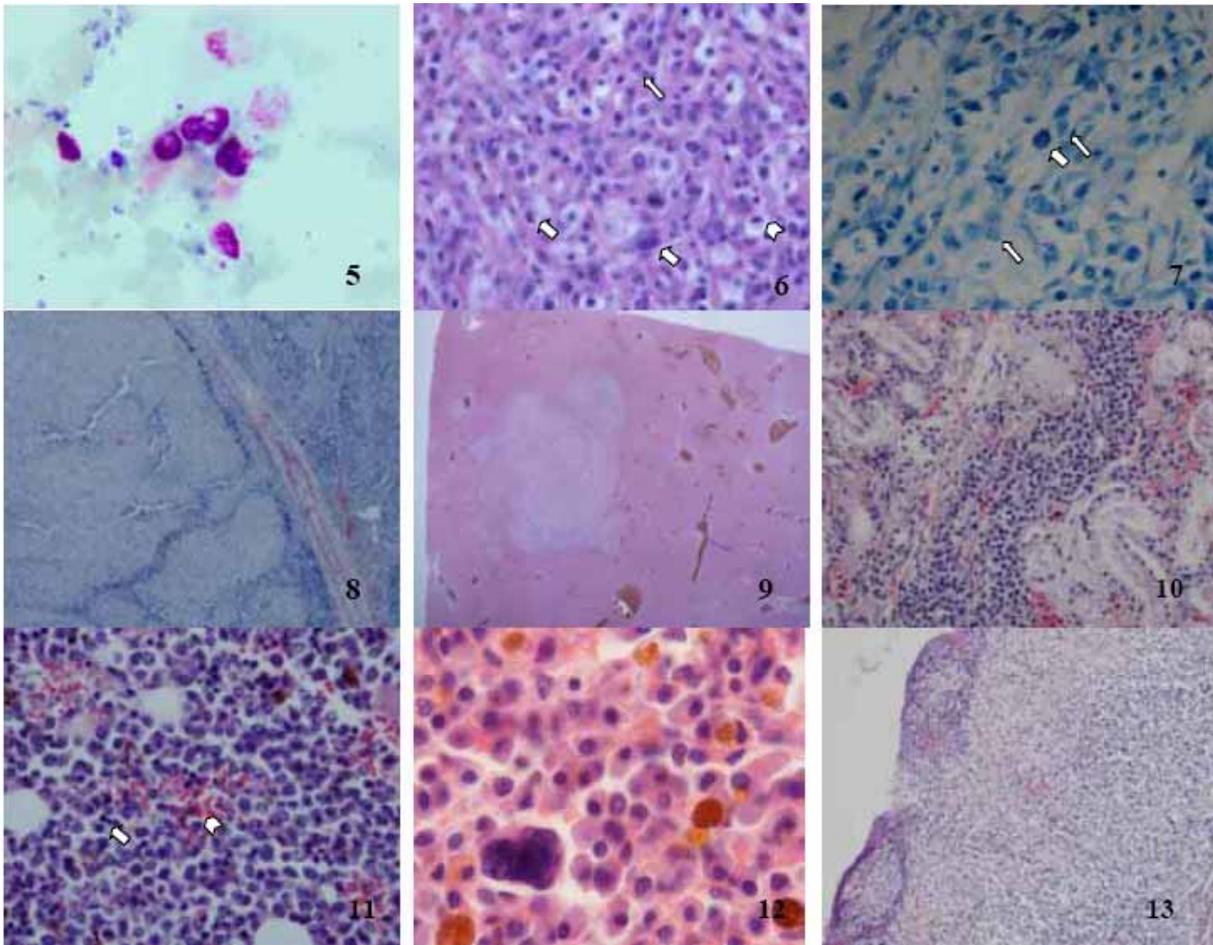
Cytological and histopathological features of DHS**. Fig. 5: Skin.** Aggregate of cells with round and folded nuclei, moderate anisokaryosis. FNA. MGG. 1000x. **Fig. 6: Skin.** Pleomorphic, round malignant histiocytes and spindle-shaped cells. Occasional vacuolation of round cells (thin arrow), high degree of anisokaryosis (two thick arrows) and mitosis (arrow head). HE. 400x **Fig. 7: Skin.** Note the absence of metachromatic granules in the cytoplasm of round cells (thin arrows), compared with a mast cell (thick aroow). Toluidine blue. 400x. **Fig. 8: Lymph node.** Severe alteration of normal architecture by infiltrating neoplastic histiocytes. HE. 400x. **Fig. 9: Liver.** Multifocal proliferation of neoplastic histiocytes replacing hepatic parenchyma. A small cluster of malignant histiocytes can be observed in the periportal area. HE. Gross magnification. **Fig. 10: Kidney.** Tumor cells and plasma cells in the interstitial tissue. Severe tubular degeneration. HE. 200x **Fig. 11: Bone marrow.** Marked infiltration by histiocytes, which have phagocyted red blood precursors (arrow) or red blood cells (arrowhead). HE. 400x. **Fig. 12: Spleen.** Histiocytes have abundant, eosinophilic and vacuolated cytoplasm. A multinucleated cell and hemosiderosis are seen. HE. 400x. **Fig. 13: Tongue.** Neoplastic histiocytes infiltrate the epithelium and deep tissues of the tongue. HE. 100x.
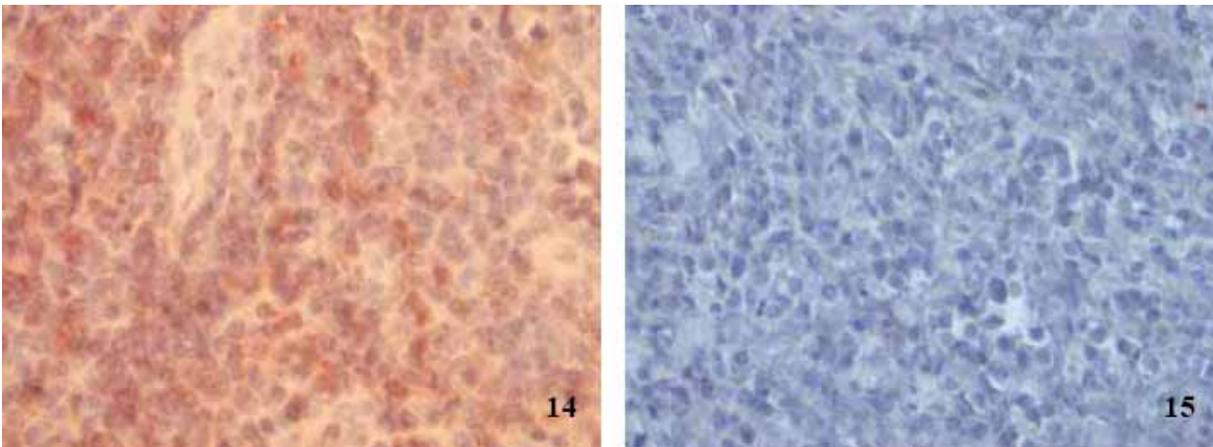
Immunohistochemical staining for CD3 of cutaneous neoplasms in DHS. **Fig. 14:** Positive control for CD3. **Fig. 15:** No visible staining of the neoplastic histiocytes with CD3, 400x.

periglomerular area (Fig. 10). Lesions consistent with chronic nephropathy were also present. The bone marrow was diffusely infiltrated with neoplastic histiocytes (Fig. 11). Neoplastic histiocytes in the *spleen* were diffusely dispersed in the red pulp. The cells had abundant, eosinophilic, frequently vacuolated cytoplasm and sharply defined borders (Fig. 12). Occasional giant multinucleated cells were observed. The epithelium of the *tongue* was diffusely infiltrated with malignant histiocytes, admixed with neutrophils and eosinophils. The infiltrate extended to the deeper tissues of the tongue (Fig. 13). No metastasis or other significant lesions were detected in other examined tissues and organs.

Mitotic index

MI counts for cutaneous neoplasms in DHS ranged from 1 to 4, with a mean and median of 2.3 and 2, respectively (Fig. 6).

Immunohistochemistry

Neoplastic histiocytes showed no visible staining with CD3 antibodies (Fig. 15).

Table1: Organs and tissues involved in DHS

Organ/tissue	Metastases yes/no	Location of metastizing cells
Liver	yes	Parenchyma, sinusoids, portal region
Spleen	yes	Diffuse
Kidneys	yes	Interstitial tissue, periglomerular area
Lung	no	-
Heart	no	-
Lymph nodes (axillary, prescapular, popliteal, mesenteric)	yes	Subcapsular sinus/diffuse
Bone marrow	yes	Diffuse
Stomach	no	-
Intestines	no	-
Pancreas	no	-
Central nervous system	no	-
Tongue	yes	Epithelium, muscular tissue

DISCUSSION

This case of disseminated histiocytic sarcoma (DHS) debuted as a periocular multifocal nodular dermatopathy and extended throughout the body in one year. At the time of the presentation, complete blood count and abdominal ultrasound examination revealed no abnormalities. Nevertheless, at the time of the necropsy, systemic spread was evident, in the form of multiple nodular lesions on tongue, liver and kidneys. Based on the history and clinicopathologic features of the case, it can be concluded that the condition debuted as a localized histiocytic sarcoma (LHS) and progressed in one year, to DHS. This conclusion is in agreement with the general belief that DHS is the result of systemic spread of LHS, rather than simultaneous development of multiple neoplasms in various organs and tissues (Affolter & Moore, 2002). In our case, the first skin nodules had an unusual location, in the periocular region. In the rare cases when the skin is the primary site for DHS, the skin nodules are located on

the extremities or adjacent to appendicular joints (Moore, http://www.histiocytosis.ucdavis.edu/sarcoma.html; Hayden *et al*., 1993; Craig *et al*., 2002; Fulmer & Mauldin, 2007).

Although the cytological examination is recognized as a reliable tool in diagnosing round cell tumors, in this case, morphological features of malignant histiocytes was misleading. One of the factors contributing to the erroneous suspicion of poorly differentiated MCT was the poor cellularity of the smear. The number of cells in the aspirate from histiocytic sarcomas are reported to vary from few (Duncan & Prasse, 1979) to abundant (DeNicola, 2008), but scientific data regarding the minimum number of cells needed for an adequate sample are limited. The few cells in the smear had morphological aspects similar to neoplastic mast cells (large vesicular nuclei, abundant cytoplasm, occasional binucleation and cytoplasmic vacuolation). The absence of granules was not a reliable feature to exclude poorly differentiated MCT, as it is known the latter can have fine granules only visible when stained with toludine blue or even lack metachromatic granules.

The final diagnosis was the result of data collected from cytology, gross examination, histopathology and immunohistochemistry. Lesions on the skin, tongue, liver and kidneys had the typical nodular appearance, but the location and gross features of the tumors were common for a large number of different neoplasms. The preliminary morphological diagnosis of a poorly differentiated cutaneous MCT, made by cytological examination, was invalidated by the absence of metachromatic granules in histological sections stained with toluidine blue. Histopathology was also instrumental in establishing the histiocytic differentiation of the process and exclusion of a number of other round cell tumors (Langerhans cell histiocytosis and melanomas), known to have different histological features (Gross *et al*., 2005). The tumor nodules consisted of a mixture of round and spindle cells arranged in loosely cohesive aggregates, with high grade of anisokaryosis, and occasional bi- or trinucleated cells. A striking similarity to MCT was seen, with respect to the distribution of metastasizing cells in the liver and kidneys. The malignant histiocytes were present in small clusters in periportal area of the liver and periglomerular and interstitial tissue in kidneys, similar to the reported location of metastasing neoplastic mast cell (Hottendorf & Nielsen, 1968). The clinical cutaneous neoplasm subjected for CD3 labeling, showed no immunohistochemical staining for this antibody. This absence of positive expression excluded the possibility of a cutaneous lymphoma.

In DHS, the presence of metastasis in lymph nodes and the internal organs associates with thrombocytopenia and anemia (Moore and Rosin, 1986). The CBC and serum biochemistry in the later stages of the disease, were unavailable in this case. This makes impossible a comparison of the hematological parameters and enzyme activity of this case with other cases of DHS reported elsewhere.

DHS is an aggressive condition, due to rapid spreading of neoplastic cells in various tissues and organs. This case reports the skin in the periocular region as a primary location for DHS, besides extremities and areas adjacent to appendicular joints. Although the importance

of cytology in diagnosis of round cell tumor has been well documented, this study illustrates the way it can also lead to mistyping of poorly differentiated round cell tumor.

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