Diagnostic value of cytological examination in canine skin tumors: A prospective study on 60 cases

M. A. TAULESCU (1), Cristina A. LELESCU (1*), Adelina L. CĂTANĂ (2), A. F. GAL (1), C. CĂTOI (1), ROXANA POPA (1)

1 Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania
2 Clinique Vétérinaire du Cèdre, Épron, 14610 France
* Corresponding author

Key words: FNAC, biopsy, histopathology, skin, tumors, dog.

Summary

The aim of this study is to determine the diagnostic accuracy of FNAC in the diagnosis of skin tumors by correlating the cytological findings with the histopathological diagnosis in a series of dogs with cutaneous masses.

Sixty cytologic and histopathological specimens were obtained from dogs with cutaneous masses and submitted for cytologic and histological evaluation; of these, 17 specimens underwent IHC analysis. Cytologic samples were obtained by fine needle aspiration technique, while histopathological samples were collected through punch and core needle biopsies. Diagnostic accuracy of FNAC was assessed by comparison with the histopathological findings.

Neoplasia was diagnosed by histology in 60 specimens; in 56.6% of cases, an accurate definitive diagnosis was reached by FNAC. Twenty-four samples (40%) were classified as malignant lesions, of which malignant melanomas were most frequently diagnosed (25%). Histiocytoma and benign perianal tumors were the most frequently encountered benign tumors, accounting for 19.44% each.

FNAC is a fairly accurate and useful diagnostic procedure to establish a definitive diagnosis in round cell and epithelial tumors of the skin; however, the necessity of histopathological evaluation in certain types of cutaneous soft tissue neoplasia is caused by its limitations in differentiating the origin of these tumors.

Introduction

Skin tumors are among the most commonly diagnosed neoplasms in veterinary medicine, not only because they are early and easily detected by owners, but also because of the constant exposure of this organ to the action of environmental factors (Couto & Moreno, 2013; Meuten, 2017). These neoplasms may have ectodermal, mesodermal and melanocytic origin (Zachary, 2016). Among the most frequently diagnosed types of skin cancer in dogs are mast cell tumors (MCTs), basal cell tumors, perianal gland adenomas, histiocytomas, melanomas, sebaceous gland adenomas and lipomas (Finnie & Bostock, 1979). A previous study showed that canine cutaneous histiocytoma was the most diagnosed type of neoplasia in a population of over 130000 insured dogs in United Kingdom (Dobson et al., 2002).

Cytological examination is a valuable diagnostic tool in veterinary oncology for obtaining useful morphological characteristics of the specimen because of its many advantages, including rapidity and ease of performance, minimal invasiveness, cost-
effectiveness and highly reliable results (Dobson & Lascelles, 2011; Withrow et al., 2013, Couto & Moreno, 2013). Due to the small quantity of the collected sample, cytological examination is not always conclusive and biopsy may be necessary (Withrow et al., 2013); although the risk is very low, dissemination of neoplastic cells can occur during fine needle aspiration (FNA) procedure. Even so, cytological examination of FNA remains an essential tool in oncological diagnosis, successfully replacing surgical biopsy in many cases (The Papanicolaou Society of Cytopathology Task Force on Standards of Practice*, 1997).

Histopathological examination of tissue biopsies is frequently required for a definitive diagnosis of neoplasia (Withrow et al., 2013). Although there are various techniques used to obtain biopsy specimens, including punch biopsy, wedge biopsy, incisional and excisional biopsy, the procedure is not risk-free; bleeding, scarring and secondary infections may occur (Nischal et al., 2008).

Immunohistochemistry (IHC) technique allows detection of specific cellular proteins by the use of fluorophore-labeled and enzyme-labeled antibodies (Idikio, 2010). A major practical advantage of this technique is that it allows the pathologist to identify the cell or tissue of origin of the neoplastic cell population. Among the frequently identified tumor markers using IHC are vimentin, cytokeratin, CD3, CD79a, CD18, desmin, S100 and melan A (Dobson & Lascelles, 2011).

The aim of this study was to evaluate the accuracy of fine-needle aspiration cytology (FNAC) in comparison to histopathological and immunohistochemical techniques in different canine cutaneous and subcutaneous tumors.

Materials and methods

For this study, cytologic and biopsy specimens were collected from client-owned dogs, brought for consultation at private veterinary clinics, but also at the Faculty of Veterinary Medicine. Dogs of different breeds and ages, both males and females, presented with cutaneous and subcutaneous solid infiltrative and/or expansive masses, met the inclusion criteria. Exclusion criteria consisted of nodular inflammatory lesions and cystic structures. Patient data including date of birth, breed, sex, neuter status and concurrent diseases was collected through Health Certificates.

Cytologic specimens were obtained by FNA, using a fine needle (22–25G) or a fine needle attached to a syringe, and by impression smear technique, mainly in the superficially located masses. Smears were prepared by squash method and blood smear technique, being subsequently stained with Diff-Quik and Wright-Giemsa.

Samples for histopathological analysis were collected by taking punch and core needle biopsies, or by using Tru-Cut and Jamshidi biopsy techniques, depending on the size, location and type of the tumor. All of the tissue sections were formalin-fixed and paraffin-embedded, and stained with hematoxylin and eosin (H&E), Alcian blue/periodic acid–Schiff (AB/PAS) or Masson trichrome staining. Microscopic analysis was performed with Olympus BX-41 digital microscope, while microscopic images were captured with a digital camera (Olympus SP 350). Tumors were classified according to Kleihues & Sobin, 2004 and Meuten, 2007.

For IHC analysis, tissue sections were mounted on poly-L-lysine coated slides and stored in a thermostat for 12 hours, at 37°C. Subsequently, immunohistochemical staining was performed with the Leica Bond Max automated immunostainer, while monoclonal and polyclonal antibodies against c-Kit, protein S100, Melan A, Pan-cytokeratin, CD34, alpha-smooth muscle actin (alpha-SMA), vimentin, CD3 and CD79 were used in order to detect the origin of the tumors.

Results and discussions

A total number of 60 dogs (female n=24; male n=36), clinical and necropsy cases, with cutaneous and subcutaneous masses was included in the present study, after cytological specimens were evaluated and met the inclusion criteria. Subsequently,
histopathological analysis was performed in all cases, while biopsies obtained from a number of 17 patients were also evaluated by immunohistochemistry (Table 1). Among dogs enrolled in the study, the following breeds were represented: Cocker spaniel (n=7), German shepherd (n=6), Husky (n=4), mixed breed dogs (n=20), Irish setter (n=3), Caniche (n=2), Doberman, Boxer, Collie, Hungarian viszla, etc. The median age of the patients was 8.6 years (range 4 months to 16 years). Specimens were collected from different body regions: perianal (n=4), thorax (n=7), ear auricle (n=3), neck (n=3), anal sac (n=5), upper lip (n=3), etc.

**Primary cutaneous round cell tumors (RCTs)**

From a total number of 16 RCTs, 14 specimens were diagnosed cytologically; however, subsequent histopathological and IHC examination was necessary in order to establish a differential diagnosis of RCTs in some cases (e.g. poorly differentiated mast cell tumors, T cell lymphoma). Complete agreement between cytopathologic and histopathologic examination was found for cutaneous histiocytoma (n=7) and non-epitheliotropic lymphoma (n=1). Mast cell tumors were diagnosed by cytology in 6 of 8 patients (75%).

Cutaneous histiocytoma was diagnosed in 4.2% (7/60) of cases, in American Staffordshire Terrier, Labrador, Schnauzer, Husky, Beagle, Chihuahua and mixed breed dogs, aged between 4 months and 13 years, both males (85%) and females (15%). The lesions were well-circumscribed, light-pink, nodular, alopecic and ulcerated, with a firm consistency, measuring approximately 1.5 cm in diameter. Highly cellular smears showing round to oval cells, with round and reniform-shaped nuclei and displaying a mild anisocytosis, were observed at cytological evaluation. Finely granular chromatin and occasional small-sized, poorly distinct nucleoli were identified. Histologically, round to oval cells, with reniform-shaped nuclei, arranged in densely sheets and numerous mitoses were noticed.

Mast cell tumors were diagnosed in 4.8% (8/60) of patients, mainly in mixed breed dogs, but also in cocker spaniel and pitbull breeds, aged between 4 and 13 years. 57% of the dogs were females, while 43% were males. Gross appearance included a variable, mainly nodular aspect of the masses, ranging between 3 to 10 cm in size, with or without an ulcerated overlying epidermis. A homogenous or multinodular aspect of white-gray color was observed on cut section. Cytological evaluation of the well-differentiated mast cell tumors revealed highly cellular smears with well-granulated, large, round or slightly oval cells. Round to oval nuclei with small nucleoli were centrally situated, and a low nuclear/cytoplasmatic (N: C) ratio was found. Unlike these, poorly-differentiated mastocytomas showed high cellular polymorphism, with large, polymorphic nuclei and sparse intracytoplasmic granules. Histologically, a proliferation of mast cells arranged in sheets or groups of variable size, infiltrated by eosinophils, was observed. Degenerated collagen (flame figures), consisting in a frayed appearance of the collagen fibrils were also noted. According to Kiupel et al., 2011, 5 out of 8 tumors were classified as high grade (malignant).

Cutaneous T-cell lymphoma was diagnosed in an 11-year-old German Shepherd male. Cytologic examination showed a moderate to highly cellular smear, consisting of a homogeneous population of lymphoid cells. Histological examination revealed a non-epitheliotropic lymphoma with superficial dermal involvement and mild epidermal atrophy, CD3+ cells being characteristic for a T-cell lymphoma. **Tumors of the hair follicle**

Follicular tumors including pilomatricoma, trichoblastoma and trichoepithelioma were diagnosed in 7 dogs.

Sensitivity of cytological examination in the diagnosis of trichoblastoma was 80%, whereas pilomatricoma and trichoepithelioma were confirmed only histologically. IHC analysis was not performed on these samples. Pilomatricoma was identified in 1.2% (2/60) of dogs, aged 5 and 8 years respectively, both females. The lesions were located on the flank and thorax; cytology was inconclusive in both cases. Histologically, well-circumscribed, cystic lesions containing basaloid cells with...
abrupt keratinization (ghost cells), admixed with keratin lamellae were identified.

**Table 1.** FNAC, histology and IHC diagnosis for 60 cutaneous tumors in dogs.

<table>
<thead>
<tr>
<th>Total number of cases/histological type</th>
<th>Cytologic diagnosis</th>
<th>Histologic diagnosis</th>
<th>IHC diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Round cell</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histiocytoma (7)</td>
<td>7</td>
<td>7</td>
<td>Not performed</td>
</tr>
<tr>
<td>T-cell lymphoma (1)</td>
<td>1</td>
<td>1</td>
<td>1, CD3+</td>
</tr>
<tr>
<td>Mast cell tumors (8)</td>
<td>6</td>
<td>8</td>
<td>Not performed</td>
</tr>
<tr>
<td><strong>Epithelial</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malignant trichoepithelioma (1)</td>
<td>0</td>
<td>1</td>
<td>Not performed</td>
</tr>
<tr>
<td>Trichoblastoma (4)</td>
<td>4</td>
<td>4</td>
<td>Not performed</td>
</tr>
<tr>
<td>Pilomatrixoma (2)</td>
<td>0</td>
<td>2</td>
<td>Not performed</td>
</tr>
<tr>
<td>Squamous cell carcinoma (3)</td>
<td>3</td>
<td>3</td>
<td>3, PanCK+</td>
</tr>
<tr>
<td>Perianal gland epithelioma (1)</td>
<td>0</td>
<td>1</td>
<td>Not performed</td>
</tr>
<tr>
<td>Perianal gland hyperplasia (1)</td>
<td>0</td>
<td>1</td>
<td>Not performed</td>
</tr>
<tr>
<td>Perianal gland adenomas (5)</td>
<td>3</td>
<td>5</td>
<td>Not performed</td>
</tr>
<tr>
<td>Perianal gland and anal sac carcinomas (2)</td>
<td>1</td>
<td>2</td>
<td>Not performed</td>
</tr>
<tr>
<td>Sebaceous gland epithelioma (1)</td>
<td>0</td>
<td>1</td>
<td>Not performed</td>
</tr>
<tr>
<td>Sebaceous gland adenoma (3)</td>
<td>0</td>
<td>3</td>
<td>Not performed</td>
</tr>
<tr>
<td>Sebaceous gland carcinoma (1)</td>
<td>1</td>
<td>1</td>
<td>Not performed</td>
</tr>
<tr>
<td><strong>Mesenchymal</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schwannoma (2)</td>
<td>1</td>
<td>2</td>
<td>2, S100+</td>
</tr>
<tr>
<td>Myopericytoma (3)</td>
<td>0</td>
<td>3</td>
<td>Not performed</td>
</tr>
<tr>
<td>Hemangioma (2)</td>
<td>2</td>
<td>2</td>
<td>2, CD31+</td>
</tr>
<tr>
<td>Hemangiopericytoma (1)</td>
<td>0</td>
<td>1</td>
<td>Not performed</td>
</tr>
<tr>
<td>Mixosarcoma (1)</td>
<td>0</td>
<td>1</td>
<td>Not performed</td>
</tr>
<tr>
<td>Leiomyosarcoma (2)</td>
<td>0</td>
<td>2</td>
<td>2, alpha-SMA+</td>
</tr>
<tr>
<td>Fibrosarcoma (1)</td>
<td>1</td>
<td>1</td>
<td>1, Vimentin+</td>
</tr>
<tr>
<td>Lipoma (1)</td>
<td>1</td>
<td>1</td>
<td>Not performed</td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cutaneous and muco-cutaneous melanomas (6)</td>
<td>3</td>
<td>6</td>
<td>6, MelanA+</td>
</tr>
</tbody>
</table>
Multifocal basophilic mineral deposits, surrounded by granulomatous reaction were also observed.

Malignant trichoepithelioma (matrical carcinoma) was diagnosed in a 12-year-old German Shepherd female. The neoplasms were located on the left forelimb.

Trichoblastoma was identified in 2.4% (4/60) of cases and no breed predisposition was noted. The lesions were seen at the level of the head and extremities. Gross features varied from small round nodules of 1 cm in diameter to large and ulcerated masses (Fig. 1A), with a white-grayish multinodular appearance on cut section. (Fig. 1B, C). Large numbers of spindle cells admixed with cubical to polyhedral cells were identified by cytological evaluation. Some groups of cells had a certain tendency of palisading and were characterized by oval prominent nuclei and a small amount of cytoplasm (Fig. 1D). The histological structure of tumors consisted of ribbons and/or trabecular structures of epithelial cells, separated by a moderate amount of connective tissue (Fig.1E).

**Fig.1.** Canine trichoblastoma A, B and C) Gross features; D) Cytological evaluation: numerous spindle and cubical cells arranged into short streams and nests; E) Trichoblastoma – mixed type (H&E stain).

**Malignant tumors of the squamous epithelium**

Squamous cell carcinoma (SCC) was cytologically diagnosed in all cases (3/60) and confirmed by histological evaluation. Lesions were located mainly on the limbs and in the inguinal region, and gross morphological alterations included multinodular, poorly demarcated, infiltrative ulcerated masses of 3 to 10 cm in diameter. Cytological evaluation revealed the presence of numerous nests composed of pleomorphic squamous cells with clear or pale acidophilic filamentous cytoplasm, pleomorphic nuclei and proeminent nucleoli (Fig. 2A, B); asynchronous maturation, binucleated cells and atypical mitotic figures were also found. Histologically, neoplastic cells arranged in irregular islets and nests were infiltrating the subjacent connective tissue (Fig. 2C, D). IHC expression of Pan-cytkteratin (CK) was present in all cases (Fig.2E).

**Fig. 2.** Squamous cell carcinoma (SCC) A, B) Cytological evaluation: clusters of pleomorphic squamous epithelial cells (DQP stain). C, D) Histological evaluation: irregular islands and nests of squamous cells with central keratinization (keratin pearls), severe desmoplasia and mononuclear inflammatory cells (HE stain). E) Positive neoplastic cells for PanCK, IHC (counterstaining with H&E).

**Perianal and tail gland lesions**

Hepatoid (perianal) and tail gland lesions were identified in 9 dogs and included: perianal gland hyperplasia, epitheliomas, adenomas and carcinomas.

Perianal gland epithelioma was diagnosed in 0.6% of dogs on the basis of histological examination, cytology being inconclusive; tumors were composed of islands and nests of small basaloid cells with hyperchromatic nuclei.

Perianal gland hyperplasia was accurately diagnosed by histology in 0.6% of cases, whereas cytology was inconclusive; dogs had gross evidence of perianal unilateral nodular masses. Histologically, the glandular cells were arranged in large lobules, surrounded by a single layer of basaloid cells and outlined by moderate amounts of connective tissue.
Regarding perianal gland adenomas, incomplete agreement between cytologic and histologic evaluation was seen. In 3 out of 5 cases, cytology revealed well-differentiated, large polygonal cells with abundant, slightly granular cytoplasm and large, round, central nuclei with small nucleoli and low mitotic activity. Histologically, the multinodular tumor was composed of well-differentiated polygonal cells and surrounded by abundant connective tissue.

Perianal gland and anal sac carcinomas were diagnosed cytologically in one out of 2 patients and confirmed histologically in both dogs. Gross appearance of perianal ulcerated, dense, infiltrative, red-gray neoplastic masses of 10 cm in diameter, with irregular shape, occasionally reaching the ventral side of the tail, was noted. Cytology showed pleomorphic, polygonal or cubical cells with pale basophilic cytoplasm, pleomorphic nuclei and multiple nucleoli. Histologically, the perianal gland was diffusely effaced by the neoplastic tissue. In anal sac carcinomas the neoplastic cells were arranged in palisades surrounding an acellular eosinophilic material (rosettes). In perianal gland carcinoma, the neoplastic islands, lobules and nests were composed of highly pleomorphic cells with pale acidophilic cytoplasm, pleomorphic, vesicular nuclei with prominent nucleoli and high mitotic activity.

**Sebaceous gland and epitrichial (apocrine) gland tumors**

Six dogs were diagnosed with tumors of the sebaceous and apocrine glands, including sebaceous epithelioma (1 case), sebaceous adenomas (3 cases), sebaceous carcinomas (1 case) and epitrichial (apocrine) carcinomas (1 case); of these tumors, only sebaceous carcinoma was accurately diagnosed through cytological examination.

Sebaceous epithelioma was diagnosed only by histological examination; the lesion was located on the chest and gross appearance included nodular alopecic masses of 2-3 cm in diameter. Histologically, severe proliferation of basal cells at the base of glandular lobules was identified.

Sebaceous adenoma was diagnosed only histologically, due to inconclusive cytology results; solitary or multiple well-circumscribed nodular lesions of 1-3 cm in diameter were detected during gross examination. The neoplastic tissue was multinodular and composed by well-differentiated epithelial cells with foamy cytoplasm and round nuclei; rare mitotic figures were observed.

Sebaceous carcinoma was correctly diagnosed by cytology, as compared with histology. An irregular, dense, infiltrative mass of 4-5 cm in diameter, with multiple areas of necrosis was located on the pinna (Fig. 3A). Cross-cutting characteristics included compact, sarcomatoid areas with a gray-white appearance and multiple areas of necrosis (Fig. 3B). Cytology showed sparse cellularity consisting of large pleomorphic cells with prominent nucleoli, basophilic vacuolated cytoplasm and numerous erythrocytes and neutrophils (Fig. 3C). Histologically, a multinodular mass composed of pleomorphic neoplastic cells, with pale, slightly vacuolated cytoplasm, pleomorphic nuclei and high mitotic activity was identified (Fig. 3D, E).

**Fig. 3.** Sebaceous carcinoma A, B) Gross appearance of the tumor; C) Cytological examination- rare pleomorphic sebocytes with vacuolated, foamy cytoplasm (arrows) (DQP stain); D, E) Histological findings of the sebaceous gland carcinoma (H&E stain).

**Peripheral nerve sheath tumors (PNSTs)**

Schwannomas were identified in 2 patients; one of the tumors was correctly diagnosed as schwannoma both through cytological and histological methods. Subsequently, these tumors were confirmed by IHC using S-100 antibody. Lesions were located on the limbs.
and gross appearance included subcutaneous, well-defined nodular masses with a multinodular, white-gray cut-surface (Fig. 4A). Cytology showed sparse cellularity and nuclear palisading; uniform, spindle cells with weakly basophilic cytoplasm and oval-shaped, fusiform and hyperchromatic nuclei were identified (Fig. 4B). Histologic appearance was characterized by the parallel arrangement of cells in highly cellular Antoni A and sparsely cellular Antoni B regions (Fig. 4C).

Cutaneous vascular and perivascular wall tumors (PWTs)
Cutaneous vascular and PWTs tumors were identified in 6 dogs and included myopericytoma, hemangiomas and hemangiopericytomas. Of these tumors, only hemangiomas were diagnosed by cytology.

Myopericytomas were diagnosed in 3 dogs (4.61%) by histological examination. Cytology revealed the presence of various groups of elongated cells, with oval nuclei, admixed with red blood cells. Histologically, concentric layers of well-differentiated, smooth myoid tumor cells were observed around the thin-walled blood vessels (Fig. 5D).

Hemangiomas were diagnosed by cytology and confirmed by histology and IHC in both cases. Groups of elongated cells, with oval-shaped, elongated and hyperchromatic nuclei, mixed with red blood cells were seen (Fig. 5A-B). Histological features of cavernous hemangioma included the presence of large, irregular blood vessels lined by well-differentiated endothelial cells, filled with red blood cells and bounded by a moderate conjunctival stroma (Fig. 5C).

A false negative diagnosis of neoplasia was established by cytology, regarding hemangiopericytoma. The lesion was located in the flank and consisted of a nodular, dense, subcutaneous well-defined red-gray mass, measuring 3 cm in diameter. Histological evaluation of myxoid hemangiopericytoma showed bundles of pericytes arranged in nodular structures.

Miscellaneous skin tumors
Other mesenchymal skin tumors including leiomyosarcomas, fibrosarcomas, myxosarcomas and lipomas were diagnosed in 5 dogs. Accuracy of FNA was 100% in the diagnosis of fibrosarcomas and lipomas, whereas myxosarcomas and leiomyosarcomas were diagnosed only by histology or immunohistochemical evaluation.

Mixosarcoma was diagnosed by histology in a 6-year-old male Husky. Leiomyosarcoma was diagnosed in 1.2% of dogs; tumor masses were located in the cervical region and near...
the temporal canthi of the eye. Cytological smears showed sparse elongated, spindle cells with elongated, centrally positioned, hyperchromatic nuclei. Bundles of elongated, well-differentiated, proliferating cells with pleomorphic nuclei and a high number of mitotic figures, oriented in various directions were seen on histological evaluation. Positive staining of alpha smooth muscle actin (α-SMA) was found by IHC.

Cytologic features of fibrosarcoma included the presence of numerous elongated, spindle tumor cells with central, oval to elongated nuclei. Histology revealed a tumor mass composed of spindle and pleomorphic cells, anachronically arranged or disposed in bundles, with numerous collagen fibers and blood capillaries.

Lipoma was diagnosed by cytological evaluation and confirmed histologically; gross morphology of lipoma consisted in a subcutaneous, well-defined nodular mass with fibrous capsule and greasy cut surface. Histological appearance included the presence of a tumor mass composed of mature adipocytes arranged in multiple lobules, outlined by a conjunctival stroma.

**Cutaneous and muco-cutaneous melanocytic tumors**

Melanocytic tumors were identified in 3.6% of cases (6/60) and included cutaneous and muco-cutaneous melanomas. 50% of these tumors (with intensely pigmented melanocytes) were accurately diagnosed by cytology, while amelanotic/poorly melanotic lesions were diagnosed exclusively by histopathological evaluation or by IHC using antibodies against Melan A. Gross appearance included nodular or multinodular white-gray to black masses, with a diameter ranging from 5 mm to 4cm. Cytology revealed the presence of pleomorphic cells with round nuclei and abundant or moderately abundant dark-brown granular cytoplasm (melanin pigment). Regarding amelanotic melanomas, prominent cellular atypia, anisokaryosis, karyomegaly and complete lack of melanin pigment were observed; binucleated cells were also present. Histologically, fusocellular (spindle) cell-type and epithelioid cell-type melanomas were identified. Fusocellular cell-type melanomas were composed of elongated cells arranged in short streams, with abundant intracytoplasmic brown-black pigment, round or oval nuclei and rare mitotic figures. Epithelioid cell-type melanomas consisted of nests of round or oval cells, with slightly acidophilic cytoplasm and scant melan granules. Positive staining for Melan A, Vimentin and S-100 protein was detected by IHC.

**Discussion**

The present study outlines the importance of FNAC in the preliminary diagnosis of canine skin tumors, which often provides a fairly accurate and even definitive diagnosis. In addition to cytological analysis, biopsies are necessary to establish a definitive diagnosis and to select the most effective treatment protocol (Bonfanti et al., 2015).

By examining a number of 60 samples from four basic types of tissue, a definitive cytological diagnosis was reached in 56.6% of the total number of histologically confirmed cases. In contrast with earlier research findings (Ghisleni et al., 2006), neoplasms of epithelial origin were the most common diagnosed tumors in the present study (41.6%), being followed by those of mesenchymal origin and RCTs.

Few studies have been published exclusively on the correlation of FNAC and histology in the diagnosis of skin tumors in dogs. Some preliminary work in this field was carried out by Griffiths et al., 1984, who evaluated a number of 147 skin tumors; a remarkable agreement between the FNAC-based diagnosis and the biopsy-based diagnosis was achieved in his study, as 105 out of 147 samples (71%) were confirmed through histological evaluation. Although there is a notable difference between the present study and the aforementioned paper regarding the agreement found between the two diagnostic procedures, there are a few similarities; a correct diagnosis of hemangiopericytoma was achieved only by histological assessment, even though cytology may be used for diagnosis when it reveals indicative features including high cellular density, groups of cohesive spindle cells and multinucleated neoplastic cells (Meuten, 2017). Still, notable differences were found regarding the diagnosis of SCC; while a
100% agreement between cytological, histological and IHC evaluations was found in our study, only 5 out of 11 tumors were accurately diagnosed by FNAC in Griffith et al., 1984 research. The ability to establish a definitive diagnose of SCC through FNAC was due to cytologic expression of evident features including anisokaryosis, binucleation, surrounding of the nuclei by a halo and a characteristic morphology of cytoplasm (Garma, 1994; Chandrashekaraiah et al., 2011).

Adnexal tumors of the skin are very common in dogs and include follicular tumors, nailbed tumors, sebaceous and modified sebaceous gland tumors, apocrine and modified acocrine gland tumors, and eccrine tumors (Goldschmidt et al., 1998). The majority of canine adnexal tumors can be easily diagnosed using a conventional histological staining; in order to determine the histogenesis of these tumors, some specific antibodies against cytokeratins, vimentin, NSE (neuronal specific enolase), and α-SMA (alpha-smooth muscle actin) are needed (Jasik et al., 2009). Canine follicular tumors are classified based on the follicular epithelial type, as: infundibular keratinizing acanthoma (IKA), trichoilemmoma, trichoepithelioma, pilomatrixoma and trichoblastoma (Goldschmidt et al., 1998).

In one study regarding the FNAC of pilomatrixoma in dogs, a correct diagnosis was reached in all of the evaluated samples, on the basis of numerous ghost cells mixed with basaloid cells with round nuclei, pale pink cytoplasm and intermediate cells with pyknotic nuclei (Masserdotti & Ubbiali, 2002). Still, the presence of ghost cells can also be seen in cystic variants of trichoepithelioma, together with cholesterol clefts and keratinous material (Meuten, 2017). Moreover, the simultaneous presence of ghost and basaloid cells did not allow us to cytologically differentiate a bening pilomatrixoma from a trichoepithelioma, and therefore a definitive diagnosis was based on histology (Albanese, 2016). Nevertheless, FNAC was inconclusive in the present study and identification of characteristic features including the presence of ghost cells, multifocal mineral deposits and granulomatous reaction was achieved exclusively by histological evaluation. However, trichoblastoma was the only hair follicle tumor accurately diagnosed by cytological analysis.

In dogs, melanoma is the most common malignant neoplasm of the oral cavity and the second most frequent subungal neoplasm (Marino et al., 1995). According to Dobson & Lascelles, 2011, only one third of canine cutaneous melanocytic tumors are malignant. Although the proportion of confirmed cutaneous and mucocutaneous malignant melanomas was high (10%), only benign cutaneous melanomas (melanocytomas) were diagnosed in other similar studies (Ghisleni et al., 2006); these tumors were accurately classified on cytology, while only 50% of our cytological results were confirmed by histology and IHC respectively. These differences are attributed to the lack or reduced number of melanin granules (amelanotic melanoma) (Cowell & Valenciano, 2013).

Round cell tumors (RCTs) are commonly reported in dogs, and include mast cell tumors, lymphomas, histiocytomas, transmissible venereal tumors (TVT) and extramedullary plasma cell tumors (Tyler et al., 2008). In a recent report (Cora et al., 2017), 19.54% of the skin tumors in dogs were diagnosed as cutaneous mast cell tumors, 11.33% as cutaneous histiocytesomas and 1.98% as cutaneous lymphomas. According to Chalita et al., 2001, mast cell tumors were the most commonly encountered type of malignant neoplasms, while lipomas were the most frequently encountered benign tumors. These findings are partially consistent with our results, given that 5 out of 24 malignancies were diagnosed as mast cell tumors, while malignant melanomas were the most frequently diagnosed malignant tumors. Contrariwise, only one lipoma was histologically confirmed in our study, while histiocytoma and benign perianal tumors were the most commonly diagnosed benign tumors. Histiocytoma was accurately diagnosed by cytological examination in all of the cases, which confirms earlier findings (Ghisleni et al., 2006; Dobson & Lascelles, 2011) with
respect to the effectiveness of FNAC in diagnosis of histiocytomas.

Conclusion

Our work has led us to conclude that the overall diagnostic accuracy of FNAC in canine skin tumors is relatively high, especially in round cell and epithelial tumors; in spite of this fact, there are some certain limitations of FNAC procedure in differentiating the origin of cutaneous soft tissue tumors.

References


