



FIRST REPORT OF CANINE MAMMARY GLAND
GRANULAR CELL TUMOUR: CASE DESCRIPTION
AND REVIEW OF THE LITERATURE

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Summary

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The case of a 5-year-old intact female Pitbull Terrier with a granular cell tumour (GCT) of the mammary gland is presented. The dog was admitted for surgical removal of a non-painful lump assumed to be a fibroadenoma. Histological and immunohistochemical findings leading to the diagnosis consisted of polygonal tumour cells with well-defined cell borders and granular cytoplasm, staining positively with neuron-specific enolase (NSE) and protein S100 and negatively for cytokeratin AE1-AE3. Some important points in the diagnostic and therapeutic approach to this uncommon neoplasm are discussed. To our best knowledge this is the first case of such tumour in the mammary gland described in the veterinary literature.

Key words: canine, granular cell tumour, mammary gland

Granular cell tumour (GCT), also known as granular cell myoblastoma, Abrikosoff's tumour, GCT of the nerve sheaths and granular cell (GC) schwannoma, is a rare neoplasm in human and animal patients. It was first described by Abrikosoff (1926). Its name comes from the granular appearance of tumour cell cytoplasm which is assumed to be related to the presence of autophago(lyso) some

(Suzuki *et al.*, 2015). From a histogenetical perspective it is considered a neoplasm with neuroectodermal differentiation, probably of the Schwannian type.

Beside its rarity, in humans, GCT is frequently localised in the breast (Dupuis *et al.*, 2009; Suzuki *et al.*, 2015). In a ten-year retrospective study of rare non-epithelial primary breast neoplasms in 1,362 human patients, GCT was diag-

nosed only in 3 cases (0.2%) (Mátrai *et al.*, 2010). Clinical presentation, X-ray mammography and ultrasonography image analysis cannot differentiate GCT from other invasive carcinomas which makes preoperative diagnosis uncertain (Nawal *et al.*, 2014; Schickman *et al.*, 2015). Radical excision with strict follow up of the patients is the common treatment given that this tumour is mostly benign and malignant variants are rarely observed.

Data on GCT in animals are scanty. To our best knowledge, the presented case is the first reported granular cell tumour located in the canine mammary gland.

According to the Histological Classification of Mesenchymal Tumors of Skin and Soft Tissues of Domestic Animals (Hendrick *et al.*, 1998), GCT is categorised within the benign subgroup of peripheral nerve tumours. The largest number of cases is described in dogs with localisation in the oral cavity. The first described case of GCT on the tongue is determined as granular cell myoblastoma (Van der Gaag *et al.* 1983) in a 9-year old Pinscher. Cytoplasmic granules produced a positive Periodic acid–Schiff (PAS) reaction, and the authors pointed out that the histogenesis of that tumour was yet unknown.

Suzuki *et al.* (2015) described nine cases of GCT on the tongue in dogs. The authors have used immunohistochemistry (IHC), digital microscopy, cultivation in a cell culture and xenotransplantation of tumour cells. In some of the cases the tumour cells presented expression of S100, CD133 and desmine, while the cytoplasmic granules were positive for LC3, p62, NBR1 and ubiquitin. The same localisation of GCT was established by Rallis *et al.*, (2001), who described a GCT case on the tongue of a 12-year old English

Pointer. IHC demonstrated positive staining for S100 and desmine and slightly positive PAS which was untypical for GCT. The authors pointed out that due to the frequent localisation of that tumour on the tongue, an IHC examination allows for its differentiation from leiomyoblastoma.

Piseddu *et al.* (2011) also described a tumour on the tongue in a 9-year old female spayed dog, initially diagnosed as GCT by a fine needle aspiration biopsy. The histology test identified multiple lipoblasts which implies liposarcoma. IHC showed a negative PAS reaction and no expression of smooth muscle actin, desmine and cytokeratin, but a positive staining for S100 and vimentin. A diagnosis of a highly differentiated liposarcoma was posed, stating that the eosinophilic granular cells were also a finding in liposarcoma and were not typical only for GCT. Kaewamatawong *et al.* (2008) reported a case of GCT on the tongue in a 9-year old male dog. The tumour cells expressly tended to form clusters divided by a delicate meshwork of reticular fibres. Mitotic figures were rare. A strongly positive PAS reaction was identified by IHC.

Another localisation of GCT was on the vocal cords in a dog (Rossi *et al.*, 2007). A single polyp was clinically identified on the left vocal cord in a 6-year old male dog. The histological examination has found out groups of globoid and polygonal cells filled with multiple diastase-negative and PAS-positive granules and scarce stroma. The described picture resembled an invasive squamous cell carcinoma. IHC, however, identified positivity to S-100 protein, CD 68, collagen IV and NSE – an evidence for GCT and its origin from the Schwann cells.

There are several reports for localisation of GCT in the peripheral and central nervous system. Rao *et al.* (2010) des-

cribed GCT in a 2-year old Great Dane with progressing paraparesis. MRI identified a large mass in the lumbar area and a second small extradural mass in the spinal canal. IHC shows a positive S100 and neuron-specific enolase (NSE) and based on that test the tumour was defined as GCT. Pérez-Klein *et al.* (2002) have identified GCT on the spinal cord in a 12-year old male dog with chronic paraparesis for a month. An extradural tumour was identified in the area of Th3-Th4. After removal, the tumour was defined as GCT by IHC. Higgins *et al.* (2001) have identified GCT in two dogs with localisation in the central nervous system. The lesions in the brain have been identified by MRI with localisation in the bulbous olfactorius and the frontal cortex, respectively. After their surgical removal a pathophysiological and IHC tests were performed and both tumours were found positive for PAS and ubiquitin, varying positive for S-100, α -1-antitrypsin, α -1-antitrypsin and vimentin and negative for glial fibrillary acidic protein (GFAP) and pan-cytokeratin. The survival time of both dogs was 4 and 12 month, respectively.

Barnhart *et al.* (2001) have also identified GCT in the central nervous system where the tumour was localised in the pituitary gland. The patient was a 12-year old Labrador Retriever with symptoms of acute blindness, convulsions, ataxia, and behavioural changes. Liu *et al.* (2004) described a case of intracranial GCT localisation in a 12-year old Miniature Poodle. A mass in the left hemisphere compressing the lateral ventricle was discovered by MRI. The IHC cells expressed a strongly positive PAS-reaction, NSE, vimentin and S-100 protein.

Mishra *et al.* (2012) identified atypical GCT on the meninges of the brain in 10-year old female Chihuahua with convul-

sions, behavioural changes and cervical pain for 2 months. MRI has identified a thickening of the meninges of the left brain hemisphere reaching the falx cerebri. After euthanasia, the histopathological examination demonstrated a cellular neoplasm with fusiform to polygonal cells with major anisocytosis and anisokaryosis. An IHC test has identified a diffuse reaction for vimentin and negative PAS. The neoplasm was defined as non-typical GCT, with an atypical infiltration course and negative PAS-reaction.

Another atypical case was that described by Pérez *et al.* (2005): a meningioma with a major component of granular cells on the left eye orbit in a 5-year old male Golden Retriever presented with exophthalmos, with the tumour formation covering the optic nerve. There were microscopic findings of clusters of round to polygonal cells with various dimensions with eccentric nuclei and a large amount of eosinophilic cytoplasm containing PAS-positive granules. The IHC neoplastic cells were positive for vimentin and less strongly for NSE and S-100 protein as well as negative for glial fibrillary acidic protein and cytokeratin. Metastases with a similar characteristic are also found in the lung and the heart.

GCT in dogs has also been reported with a different localisation. Lu & Dubielzig (2012) described eight cases of GCT on the eyelids in dogs. In all cases the mass was localised on the eyelid conjunctiva in the area of the median cantus. A positive PAS-reaction was identified in all eight tumours while seven of them did not relapse after removal which evidenced their benign nature. According to the authors GCT on the median cantus should be considered with respect to the neoplasms on the eyelids in dogs.

Sanford *et al.* (1984) described a primary GCT of the heart in a 9-year old dog. The tumour involved the right auricle. A PAS-reaction of varying positivity was histologically identified. GCT was identified in an adult German Shepherd dog with localisation in the pleura, the lung, the mediastinum and the diaphragm (Foley, 1988). Histopathologically, the mass consisted of rounded cells with eosinophilic, weakly PAS-positive cytoplasmic granules.

Patnaik (1993) described retrospectively GCT in 6 dogs, 3 cats, one horse and a parrot. Five of the tumours in the dogs were localised in the oral cavity and one on the meninges. The three tumours in the cats were on the tongue, vulva and finger, respectively. The tumour in the horse was in the lung and was discovered during necropsy, while that in the parrot was on the periocular soft tissues. The tumour on the finger in the cat was defined as malignant. All tumours, with the exception of that in the parrot gave a positive reaction for vimentin. The equine tumour was positive for S100 and NSE, and the malignant tumour on the feline finger and that in the parrot were positive for actin and only that in the parrot – for desmine.

Kelley *et al.* (1995) also identified

GCT in six horses, again in the lung. In five horses the masses were multiple, and in the sixth – single, white and solid node involving the bronchi and bronchioles. Histopathologically, the neoplastic cells were round or angular with eosinophilic cytoplasmic granules positive for S-100 and NSE.

Irizarry-Rovira (2008) described a rare and so far unique GCT case on the testicle in a pet rabbit (*Oryctolagus cuniculus*). After removal of the testicle a histopathological, IHC and digital microscopic test was performed. The authors noted the rare and not described so far localisation of GCT and the need of differential diagnosis with the other testicle tumours and, in particular with Leydigoma due to the great histopathological similarity.

Case presentation

A 5-year old female intact Pit Bull Terrier was presented with a slowly growing oval soft lump (4/3 cm) of the third right mammary gland (Fig. 1). The lump was painless, mobile and non-adherent to the skin and muscles. The axillar and inguinal lymph nodes were not enlarged. Blood counts, biochemical profile and electrolytes were within the normal range. X-ray examination did not detect any pathological formations in the lungs. The constella-



Fig. 1. Nodular mass in the mammary gland.

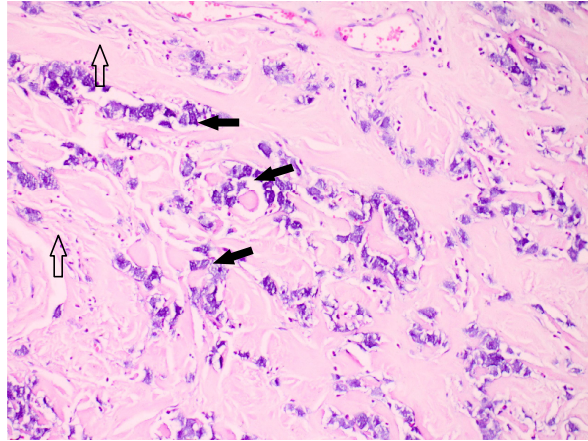


Fig. 2. Tumour mass, composed of polygonal poorly cohesive tumour cells (black arrows) with granular basophilic cytoplasm dispersed in desmoplastic stroma (white arrows) (hematoxylin-eosin, 200 \times).

tion of findings resulted in a fibroadenoma as a preoperative clinical diagnosis and mastectomy of the affected mammary gland with ovariectomy was performed.

The obtained surgical material was fixed in 4% formalin, then routinely processed, paraffin-embedded, sectioned, and stained with haematoxylin and eosin (H&E) in Leica Autostainer XL. Special staining with Periodic Acid – Schiff (PAS) was manually performed.

Ancillary immunohistochemical (IHC) staining was performed using the standard avidin-biotin immunoperoxidase method. Antigen retrieval was performed by the automated system PT-Link (DAKO) using a citrate buffer (10 mmol/L, pH 6) for Neuron-Specific Enolase (NSE) (RTU, Clone BBs/NC/VI-H14) and an EDTA buffer (pH 9) for Cytokeratin (RTU, Clone AE1-AE3), CD 68 (1:100, Clone PG-M1), S100 (1:400, Polyclonal), Ki67 (RTU, Clone MIB-1) and p53 (RTU, Clone DO-7). An Antigen-Antibody complex was visualized by a 3,3'-diaminobenzidine (DAB)-based detection

system (EnvisionTMFLEX).

Histological findings consisted of mammary gland tissue infiltrated by polygonal tumour cells with distinct cell borders, basophilic granular cytoplasm, and round hyperchromatic nuclei. Mild cellular and nuclear polymorphism was observed. No detectable mitoses and necrosis were found. The cells were dispersed in desmoplastic stroma with a scant inflammatory component and demonstrated a vague tendency to form aggregates, sheets, and linear structures (Fig. 2). The usage of histochemical stains verified the presence of PAS-positive cytoplasmic granules.

Immunohistochemical study revealed a constantly positive and diffuse cytoplasmic reaction with S100 protein (Fig. 3) and neuron-specific enolase – NSE (Fig. 4) in the neoplastic tissue. Single tumour cells stained with CD 68. Cytokeratin AE1-AE3 was negative. Proliferative index Ki67 did not exceed 10%. A peculiar granular cytoplasmic expression of p53 was observed. It was an expected finding since such a shift of this typically

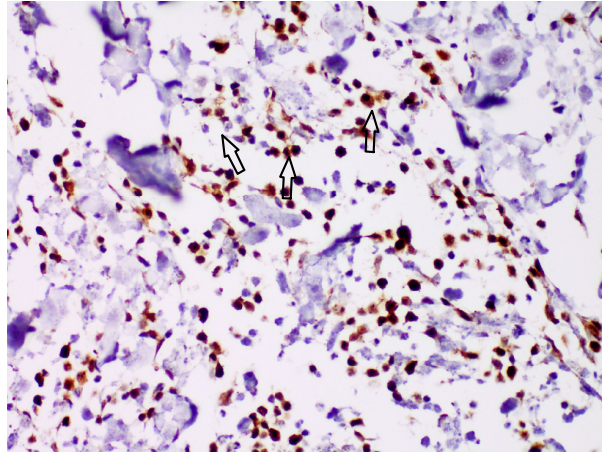


Fig. 3. Immunohistochemical staining for S100 protein – intensive expression (arrows) in tumour cells (200×).

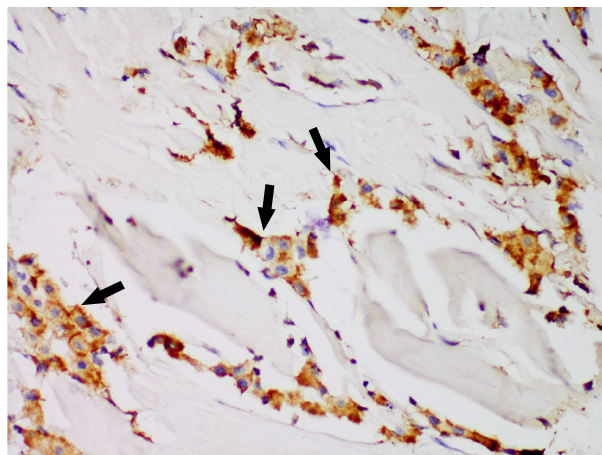


Fig. 4. Immunohistochemical staining for NSE – positive reaction (arrows) in tumour cells (200×).

nuclear marker has never been reported in granular cell tumours. In humans, the latter phenomenon was described in some breast carcinomas and in neuroblastomas. In GCTs it needs a more profound analysis since the evaluation of nuclear p53 expression is one of the components with great impact on the biological behaviour

of these neoplasms.

The differential diagnosis in this case included malignant epithelial neoplasms – primary carcinoma of the mammary gland, malignant melanoma, mast cell tumour and histiocytic proliferative disorders – histiocytoma and cutaneous histiocytosis. The pathological conclusion of GCT was

a constellation of 1) histological features – polygonal tumour cell with granular cytoplasm within fibrotic stroma; 2) histochemical stain patterns – PAS+ cytoplasmic granules and 3) the immunohistochemical profile – S100+/NSE+/CD68±/CKAE1-AE3 (-).

Routine markers that aid the exclusion of other possible neoplasms are: CKAE1-AE3 – expressed in carcinomas, CD117 – stains mastocytes, CD1 – positive in clonal histiocytic proliferations, HMB45, MelanA in melanomas. The latter can be found focally expressed in some GCT. Given their mutual neuroectodermal histogenesis, there is a significant overlap between the immunohistochemical profiles of GCT and malignant melanoma (S100+/HMB45±/MelanA±) defining the quest for more specific markers both in IHC and molecular biology.

Regarding the few cases described in the literature there are still no certain markers of the biological behaviour. In human population, Fanburg-Smith *et al.* (1998) proposed the following six histological criteria to determine whether a tumour is malignant or not: 1) necrosis; 2) spindling; 3) vesicular nuclei with large nucleoli; 4) increased mitotic activity (> 2 mitoses/10 high-power fields at 200× magnification); 5) high nuclear to cytoplasmic (N:C) ratio and 6) pleomorphism. If none of these diagnostic criteria are met, the tumour is considered benign. If one or two criteria are met, the tumour is considered atypical, and if three or more criteria are met, the tumour is considered malignant. A proliferative index Ki67 >10 % and p53 expression in 50% or more of the tumour cells are considered bad prognostic factors. The authors report that the risk of local metastases in benign GCTs is 2–8% and up to 32% in malignant cases with clinical manifestation in the first

year.

As a rare neoplasm GCT tends to pose even more questions than answers. With regard to adjuvant treatment there is no established protocol which makes surgical removal of the tumour with clinical follow-up the most common approach for this nosology. In this case, two year after the surgery the dog shows no signs of progression.

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