NEOPLASTIC DISEASE

Short Title: Triton Tumour in a Cat

Malignant Cutaneous Peripheral Nerve Sheath Tumour with Rhabdomyosarcomatous Differentiation (Triton Tumour) in a Domestic Cat

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Summary

Divergent differentiation is encountered frequently within human malignant peripheral nerve sheath tumours (MPNSTs). The new component is often a rhabdomyosarcoma, but in animals this specific form of divergent differentiation within MPNST has only been reported once (in a dog). Incisional wedge biopsy of a locally extensive, ventral abdominal wall mass, which extended from the dermis to the subcutis, from a 12-year-old female domestic short haired cat, was performed. The tissue was examined with routine haematoxylin and eosin staining and immunohistochemical methods. A malignant neoplasm with spindle and polygonal cell components and progression towards a rhabdomyosarcomatous phenotype was observed.   Both neoplastic cell populations exhibited strong expression of vimentin and there was multifocal expression of S100 and desmin. There was strong cytoplasmic labelling for α-sarcomeric actin and muscle actin, and weak labelling for myoglobin within the cells positive for desmin. There was multifocal positive nuclear labelling for myogenin. Glial fibrillary acidic protein, α-smooth muscle actin, microphthalmia-associated transcription factor and melanoma antigen recognized by T-cells were not expressed. Microscopical features, aided by immunohistochemistry, identified a MPNST with progression towards a rhabdomyosarcomatous phenotype, a so-called 'triton tumour'. A Schwann cell component could account for the divergent patterns of growth, given the plasticity of the neural crest. Nerve sheath tumours have been reported in the skin and subcutis of cats and are a differential diagnosis of feline cutaneous spindle cell neoplasms.

Keywords: triton tumour; cat; peripheral nerve sheath tumour; rhabdomyosarcomatous differentiation

Peripheral nerve sheath tumours (PNSTs) are neoplasms composed of single or mixed populations of Schwann cells, perineurial cells and intraneural fibroblasts of the peripheral nervous system (Schulman *et al.*, 2009). In veterinary medicine, their classification is often simplified into benign and malignant forms (Hendrick, 2017). The human classification recognizes three major benign types: neurofibromas, schwannomas and perineuriomas; all have malignant counterparts, of which neurofibrosarcomas and malignant schwannomas are the most important. Because the malignant variants typically lack the microscopical differentiating features which are the hallmarks of their benign counterparts, they are now simply designated malignant PNSTs (MPNSTs). Human MPNSTs may contain areas of 'divergent differentiation', the development of a second and different malignant lineage within the primary nerve sheath neoplasm, most often into a skeletal muscle sarcoma, but angiosarcomatous, chondrosarcomatous, osteosarcomatous and carcinomatous patterns are also recognized (Ducatman and Scheithauer, 1984). The term 'triton tumour' for the rhabdomyosarcomatous variant follows the work of Locatelli, in which supernumerary limbs containing muscle and bone were induced to grow following the implantation of sciatic nerve into soft tissues of the back of triton salamanders (Locatelli, 1925; Stasik and Tawfik, 2006). Masson (1932) first described the presence of rhabdomyosarcomatous elements within MPNSTs from patients with neurofibromatosis. He theorized that either neoplastic Schwann cells were capable of inducing muscle differentiation of other endoneurial cells or, being of neural crest origin, Schwann cells could undergo mesenchymal differentiation during malignant transformation (Masson, 1932). As the neural crest contributes to craniofacial mesodermal structures during normal development, Masson's latter thesis seems sound.

Benign neurofibromas, schwannomas and a few perineuriomas are known in animals, but detailed reports are few (Higgins *et al.*, 2006; Ochi *et al.*, 2008; Schoniger and Summers,

2009; Schoniger *et al.*, 2011). PNSTs in animals occur most commonly in the dog and most of these are MPNSTs, arising within the brachial plexus, other spinal nerves or the trigeminal nerve (Summers *et al.*, 1995). A comprehensive 2009 report described 59 PNSTs in 53 cats, which represents the largest study of these tumours in this species (Schulman *et al.*, 2009). In this large series, in contrast to the dog, 75% of tumours involved the skin, subcutis, skeletal muscle and/or mucous membranes and 16 tumours (27%) demonstrated features of malignancy. In none was divergent differentiation identified, and all evaluated (59/59) were negative for skeletal muscle markers.

This report describes a malignant triton tumour in a 12-year-old female domestic shorthaired cat. The cat presented to the referring veterinarian with what was described as an extensive (at least 15 × 8 cm) subcutaneous mass on the ventral abdomen that had 'become larger recently'. An incisional wedge biopsy sample of the mass (approximately 3 × 4 × 2.5 cm) was surgically removed and submitted for histopathological examination.

The tissue was fixed in 10% neutral buffered formalin, processed routinely and embedded in paraffin wax. Sections (4 μm) were stained with haematoxylin and eosin (HE).

Immunohistochemistry (IHC) was performed using the EnVision system (Dako, Stockport, UK). Briefly, endogenous peroxidases were blocked with peroxidase-blocking solution for 15 min at room temperature. Antigen retrieval was performed using citrate antigen unmasking solution (Vector Laboratories, Peterborough, UK) in a steamer for 15 min. Slides were incubated with primary antibodies (for 30 min) specific for: vimentin (Dako; 1 in 500 dilution), S100 (Novocastra, Milton Keynes, UK; 1 in 500 dilution), glial fibrillary acidic protein (GFAP; Novocastra; 1 in 100 dilution), desmin (Novocastra; 1 in 100 dilution) and α-smooth muscle actin (α-SMA; Novocastra; 1 in 100 dilution). 3, 3'-diaminobenzidine tetrahydrochloride (DAB) was used as chromogen and sections were

counterstained with haematoxylin. As a negative control, a duplicate of each section was incubated with normal serum from the same species as the primary antibody.

Further IHC was performed employing antibodies specific for: pan-muscle actin (Dako; 1 in 400 dilution with no pre-treatment), α-sarcomeric actin (Enzo, Farmindale, New York, USA; 1 in 40 dilution with microwave EDTA pre-treatment) and myoglobin (Enzo; 1 in 4,000 dilution with microwave EDTA pre-treatment). This was followed by the application of three further antibodies in order to evaluate whether there was expression of microphthalmia-associated transcription factor (MITF), melanoma antigen recognized by T-cells (MART-1) and myogenin (Myf-4). The first two of these reagents are melanoma markers and the third identifies myogenic cells.

Histopathological examination revealed a tumour, located predominantly within the subcutis and deep fascia, which multifocally infiltrated and effaced the panniculus muscle, dermis and dermal appendages, encircling hair follicles. The epidermis was multifocally raised by the underlying neoplasm and extensively ulcerated. The tumour exhibited a mixture of compact and looser, myxoid patterns of growth, with spindle-shaped tumour cells in the former and spindle-shaped to polygonal cells in the latter (Fig. 1). It contained densely cellular compact fascicles of slender spindle-shaped cells, which sharply abutted areas where the tumour growth was less dense, myxoid and vacuolated. Bundles of spindle-shaped cells had slender fusiform nuclei with finely clumped chromatin and elongated eosinophilic cytoplasmic processes, often arranged in a storiform or 'herringbone' pattern. This alternated with large lobules of more loosely arranged, shorter spindle-shaped to polygonal cells, which had oval to fusiform nuclei with variable chromatin content and amphophilic cytoplasm within an abundant myxoid stroma. Multifocally, there were large foci of tumour necrosis and the mitotic rate of the neoplastic population was 22 per ten ×400 fields in the compact

areas and 9 per ten ×400 fields in the areas with polygonal cells. Located predominantly in areas of transition between densely cellular fascicles and more myxoid areas, small numbers of plumper cells contained more abundant and deeply eosinophilic cytoplasm and 1–4 oval to fusiform nuclei with conspicuous eosinophilic nucleoli (Fig. 2). An initial diagnosis of a biphasic spindle cell sarcoma was reported. The first round of IHC employed vimentin, S100, GFAP, α-SMA and desmin antibodies. There was diffuse, strong expression in both cell populations of vimentin and multifocal cytoplasmic and nuclear labelling for S100 (Fig. 3). Multifocally, within the looser, more myxoid areas, strong cytoplasmic labelling with desmin was observed in single and multinucleated neoplastic cells, suggesting recapitulation of myogenesis (Fig. 4). In these latter areas, labelling for S100 was much less apparent. There was no expression of α-SMA or GFAP. With subsequent IHC, there was strong cytoplasmic labelling with α-sarcomeric actin within those cells positive for desmin. The cytoplasm of these cells was also weakly positive for myoglobin. Approximately 10% of cells were strongly positive for pan-muscle actin with intense cytoplasmic labelling in the subset of cells with more abundant cytoplasm (shown in Fig. 2). Further IHC for Myf-4, gave positive nuclear labelling in more myxoid areas, while MiTF and MART-1 were negative. Given the expression of both neural and myogenic markers by this largely undifferentiated spindle cell neoplasm, a triton tumour was diagnosed.

Benign and malignant tumours of the peripheral nerve sheath are recognized in several domestic, laboratory and exotic animals and are most common in dogs (Summers *et al.*, 1995). They arise at a variety of sites including the cranial, spinal and peripheral nerves, skin/subcutis and mucous membranes. In the cat, PNST typically presents as soft tissue masses (Jones *et al.*, 1995; Schoniger and Summers, 2009). The soft tissue group present the greater diagnostic challenge and must be differentiated from a number of mesenchymal

spindle cell tumours such as fibroblastic, myofibroblastic and smooth muscle neoplasms. The last two may express smooth muscle actin or desmin, but none of the three are positive for S100. GFAP antibody has been found to label some normal Schwann cells and some PNSTs. In a study of 22 benign schwannomas, GFAP was positive in 6/6 tested with up to 75–90% of the neoplastic cells labelled (Schoniger *et al.*, 2011). The failure to label this malignant PNST is not surprising, as mentioned above and described by Schoniger *et al*. Schwannomas and a variety of neurofibromas have been identified in several species (Schoniger and Summers, 2009; Schoniger *et al.*, 2011) and probably occur in the cat. In the largest series of feline PNSTs published (Schulman *et al.*, 2009), they were classified only as benign or malignant, although some of the microscopical patterns of growth described (i.e. Antoni A, Antoni B, Verocay bodies) would imply that some were schwannomas.

This cutaneous mass was interpreted as being a malignant PNST, and its mixed nature, evident in HE-stained sections, reflects a tumour containing undifferentiated neoplastic cells mixed with areas with progression towards a rhabdomyosarcomatous phenotype. Based on human experience with this entity (triton tumours are well known in man; Fletcher, 2007), the interpretation is that both undifferentiated and differentiated components are derived from one PNS cell type, and S100 expression (in both primitive and differentiated components) points to this being a Schwann cell. Cells suggesting progression towards a rhabdomyosarcomatous phenotype (myoblast like) were identified in HE-stained sections and this interpretation was supported by immunohistochemical evidence of rhabdomyoblastic development. Employing markers expressed across skeletal muscle development also allows neoplastic cells with early progression towards a myogenic phenotype to be identified among a background of otherwise undifferentiated cells (Fig. 4).

The capacity of human MPNSTs to undergo divergent differentiation into sarcomatous or carcinomatous tissue is well known (Stasik and Tawfik, 2006). Rhabdomyoblastic differentiation is the most frequently encountered pattern of divergent differentiation within a human MPNST and malignant triton tumours are composed of typical undifferentiated MPNST elements with focal areas containing rhabdomyoblasts, which can be identified by light and electron microscopy and by IHC. While divergent differentiation within MPNST has also been recognized in the dog, with carcinomatous, chondrosarcomatous and osteosarcomatous components (Summers *et al.*, 1992; Patnaik *et al.*, 2002; Schoniger and Summers, 2009;), rhabdomyosarcomatous change has been reported only once as far as we are aware. This case report describes rhabdomyosarcomatous elements in a cervical (C5–C6) spinal nerve root tumour from a giant Schnauzer dog (Dahme *et al.*, 1987). One study of canine MPNST found that non-specific myoglobin labelling can be found in up to 64% of such cases (Chijiwa *et al.*, 2004). Of the 59 PNST reported in cats, (Schulman *et al.*, 2009), in none was divergent growth identified, while 16/59 were viewed as malignant. In man, malignant triton tumour has a poor prognosis and histopathological examination is not able to predict outcome, therefore cases are followed for as long as possible after treatment (Gao *et al.*, 2015). Malignant triton tumours have a poorer prognosis than MPNSTs, which has been linked to a high frequency of grade III tumours in this variant (Brooks *et al.*, 1985). Following diagnosis, and based on our knowledge of the malignant behaviour of triton tumours in man, the owner elected to humanely destroy the cat.

Having not previously encountered a triton tumour, we were uncertain how to interpret this extensive and deeply infiltrative cutaneous spindle cell tumour, which showed both neural and myogenic differentiation. Because of this uncertainty, the experience of the

medical profession was invaluable. The novel case reported herein broadens the spectrum of

spontaneous neoplasms in animals and, in particular, soft tissue spindle cell tumours of cats.

Conflict of Interest Statement

The authors declare that they have no conflict of interest with respect to the publication of

this manuscript.

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Figure Legends

Fig. 1. The neoplasm expands the dermis and infiltrates among adipocytes. On the right are compact undifferentiated spindle-shaped cells. On the left are fascicles with plump spindle-shaped cells surrounding larger neoplastic myoblastic cells. HE. Bar, 50μm.

Fig. 2. Multifocally admixed with the spindle cell population are small numbers of larger neoplastic cells with more abundant, deeply eosinophilic cytoplasm and enlarged oval to fusiform, open-faced nuclei with prominent eosinophilic nucleoli; a few are multinucleated. HE. Bar, 50μm.

Fig. 3. S100 antibody labels clusters of neoplastic cells within the undifferentiated parts of the tumour, in the cytoplasm and, in a few cells, the nucleus also. IHC. Bar, 30μm.

Fig. 4. Desmin antibody labels the cytoplasm of individual and fused rhabdomyoblastic neoplastic cells; the latter line up, recapitulating myogenesis. IHC. Bar, 25μm.