J. Comp. Path. 2014, Vol. 150, 361–365

Available online at www.sciencedirect.com

ScienceDirect

ELSEVIER

www.elsevier.com/locate/jcpa

NEOPLASTIC DISEASE

Peripheral Neuroblastomas in Dogs: A Case Series

A. M. Arenas-Gamboa*,†, M. Tanabe‡, J. Edwards‡ and R. Storts†

** Department of Veterinary Pathobiology, Texas A&M University, College of Veterinary Medicine and Biomedical Sciences, † Department of Molecular and Cellular Medicine, College of Medicine, Texas A&M University, Health Science Center, College Station, TX and ‡ Antech Diagnostics, Irvine, CA, USA*

Summary

The peripheral neuroblastic tumours (PNTs) include neuroblastoma, ganglioneuroblastoma and ganglioneuromas. These subtypes reflect a spectrum of differentiation of progenitor cells of the sympathetic nervous system from tumours with predominant undifferentiated neuroblasts to those consisting of neuronal cell bodies that are well differentiated. Peripheral neuroblastoma is a tumour composed of neuroblastic cells with no or limited neuronal differentiation. In dogs, peripheral neuroblastoma is rare. The present report documents nine cases of canine peripheral neuroblastoma, the majority occurring as large masses in the craniodorsal abdominal cavity of young dogs (mean age of 3 years at diagnosis). Microscopically, all of the masses consisted of round to oval cells with a scant cytoplasm and hyperchromatic nuclei. Homer-Wright rosettes and pseudorosettes were evident in three of the nine cases. Neoplastic cells were immunoreactive in varying degrees to S100, neuron-specific enolase, synaptophysin, chromogranin A, tyrosine hydroxylase (one case) and were negative for vimentin, cytokeratin, CD3 and CD79a, indicating a neurogenic nature. Four of the nine cases occurred in Labrador retrievers (44%) and two (22%) in boxers, suggesting a possible breed predisposition.

Keywords: dog; Labrador retriever; neoplasia; peripheral neuroblastoma

Introduction

Neuroblastomas can occur in the central and peripheral nervous systems. Several different designations have been used to identify peripheral neuroblastoma or neuroblastoma-related tumours, resulting in some confusion regarding the terms used. In this report, the classification proposed by the Armed Forces Institute of Pathology (Koestner *et al*., 1999) is used. The peripheral neuroblastic tumours (PNTs) include neuroblastoma, ganglioneuroblastoma and ganglioneuromas. These subtypes reflect a spectrum of differentiation of progenitor cells of the sympathetic nervous system ranging from tumours with predominantly undifferentiated neuroblasts to those consisting of neuronal cell bodies that are well differentiated (Park *et al*., 2008). By definition, a neuroblastoma is a tumour composed of neuroblastic cells forming groups or nests with no or limited Schwann cell proliferation (Jiang *et al*., 2011). In man, peripheral neuroblastoma is the most common neoplasm of childhood, but its incidence in domestic animals is unknown with only a few reports documented in the dog and cow (Frye and Clement, 1970; Louden *et al*., 1992; Forrest *et al*., 1997; Matsushima *et al*., 1998; Uchida *et al*., 1998; Capucchio *et al*., 2003; Suzuki *et al*., 2003; Marcotte *et al*., 2004; Steinberg *et al*., 2006). Reports in dogs include cases described in two Labrador retrievers (2 and 3 years of age), one boxer (1.5 years old), one English setter (1.5 years old), one German shepherd dog (1 year old) and one beagle.

The clinical presentation of neuroblastomas depends on the site of tumour origin, which can be anywhere within the sympathetic nervous system. In children, the majority of these tumours occur in the abdomen, with over 50% arising in the adrenal gland. Other common sites include the neck, pelvis and chest (Park *et al*., 2008). In dogs, this tumour has been reported to occur in the abdominal cavity, adrenal gland and mandibulopharyngeal area. Some canine cases have multiple organ involvement including

Correspondence to: A. M. Arenas-Gamboa (e-mail: aarenas@cvm.tamu.edu).

0021-9975/$ - see front matter
http://dx.doi.org/10.1016/j.jcpa.2013.11.211

the kidneys, liver and mesenteric lymph nodes (Matsushima *et al.*, 1998; Suzuki *et al.*, 2003; Marcotte *et al.*, 2004). The present report documents a series of nine cases of peripheral neuroblastoma in dogs.

Materials and Methods

Animals

Between May 2003 and June 2009, nine cases (seven biopsy samples and two cases with complete necropsy examination [cases 5 and 7]) of neuroblastoma were referred to Antech Diagnostics or to the Texas A&M University Veterinary Teaching Hospital. Clinical information was obtained from accompanying biopsy/necropsy submission forms and follow-up telephone conversations with the attending clinician. In the case of the biopsy submissions, the owners did not return to the submitting clinics for further treatment of their animals and no follow-up information was obtained. Dogs were between 11 months and 10 years old, with a mean age of 3 years. Four were Labrador retrievers, two boxers and three were mixed breed dogs. Five were females and four were males.

Histopathology

Tissue samples were fixed in 10% neutral buffered formalin, processed routinely and embedded in paraffin wax. Sections were stained with haematoxylin and eosin (HE). Formalin-fixed and paraffin wax-embedded tissues were also evaluated by immunohistochemistry (IHC). Selected sections were subjected to the streptavidin–biotin–immunoperoxidase technique using primary antibodies specific for S100 protein (Dako Corporation, Carpinteria, California, USA; rabbit polyclonal), neuron-specific enolase (NSE; Dako; mouse monoclonal), chromogranin A and B (Dako; rabbit polyclonal), cytokeratin (Dako; rabbit polyclonal), vimentin (Dako; mouse polyclonal), tyrosine hydroxylase (Dako; mouse polyclonal), synaptophysin (Dako; mouse monoclonal) and in some cases CD3 and CD79a (Dako; mouse polyclonal). Antibodies were used at a 1 in 1,000 dilution. Canine brain tissue was used for a positive control and the primary antibodies were omitted for a negative control.

Results

Clinical Presentation

Clinical signs were variable and non-specific and included lethargy (cases 1, 2 and 4–7), anorexia (cases 1, 2, 4–7 and 9), diarrhoea (cases 6, 7 and 9), vomiting (cases 6 and 9), excitability (case 7) abdominal palpable masses (cases 1 and 2) and in one case (case 7) episodes of epilepsy over the previous year (Table 1).

Table 1
Clinical presentation of peripheral neuroblastomas

Case number	*Breed/sex*	*Age*	*Clinical signs*	*Location*
1	Labrador retriever/male	8 years	Anorexia, lethargy, palpable abdominal mass	10 kg, abdominal mass adherent to omentum, liver, pancreas and stomach
2	Pomeranian mixed breed/female	1 year	Anorexia, lethargy, palpable mass in right caudal abdomen	Large abdominal mass adherent to the dorsal aspect of the pelvic inlet
3	Mixed breed/male	11 months	Palpable mass along the left jugular furrow	Large mass along the left jugular furrow
4	Labrador retriever/ neutered male	1 year	Three day history of lethargy and anorexia	Multiple masses: (1) 10 × 8 cm mass adherent to the aorta and extending to the epaxial muscle; (2) large abdominal mass
5	Boxer/female	1 year	Anorexia, lethargy	Large abdominal mass firmly adherent to the dorsal aspect of the abdominal wall (necropsy examination)
6	Labrador retriever/ neutered female	2 years	Anorexia, lethargy, diarrhoea vomiting, renal failure	Abdominal mass firmly adherent to the kidneys and omentum
7	Boxer/neutered female	2 years	1-year history of seizures, lethargy, anorexia; diarrhoea for the last week	25 × 20 × 15 cm multilobulated abdominal mass adherent to kidneys, adrenal glands, small intestine and diaphragm (necropsy examination)
8	Labrador mixed breed/ neutered male	10 years	Haemoabdomen	Rupture peritoneal mass
9	Mixed breed/female	2 years	2-week history of anorexia, diarrhoea vomiting and painful abdomen	Large abdominal mass firmly adherent to the mesentery and multiple hepatic masses

Gross Lesions

Abdominal exploratory surgery or necropsy examination revealed in all cases large masses (up to 10 kg) usually along the craniodorsal aspect of the abdominal cavity and involving one or more of the omentum (cases 1 and 6), liver (cases 5 and 7) pancreas (case 1), stomach (case 1), aorta (case 7), adrenal glands (case 7), diaphragm (case 7) and small intestine (case 7). In one necropsy case, the masses occupied primarily the left side of the abdominal cavity and encompassed several loops of the small intestine, the cranial poles of both kidneys, the liver, the adrenal glands and the surface of the caudal vena cava (without extension into the lumen) and the left crus of the diaphragm (Fig. 1). On cut surface, the masses had a multilobulated pattern with smooth, tan to white, moderately firm nodules that contained multifocal, dark to brown friable areas (necrosis). In two cases the site of origin could not be determined due to the extent of the masses (cases 5 and 7). Gross evidence of lymphatic involvement was present in only one case (case 7).

Microscopical Lesions and Immunohistochemistry

Microscopical features of the masses were an infiltrative, unencapsulated, densely cellular tumour composed of sheets of round to oval cells with distinct cell borders except in areas where there was closely aggregated, scant and lightly eosinophilic cytoplasm, round hyperchromatic nuclei with finely stippled chromatin and indistinct nucleoli (Fig. 2). In all of the cases the mitotic index was high (mean 4–10 mitoses per ×40 objective field). Three of the nine cases had typical Homer-Wright rosettes characterized by central granular to fibrillar eosinophilic material surrounded by rows of neoplastic cells with variably visible thin cytoplasmic processes directed centrally or pseudorosettes, in which neoplastic cells surrounded blood vessels (Fig. 3). Admixed within the neoplastic cells were large foci of necrosis and haemorrhage. When present, metastatic lesions in other organs had similar morphology and arrangement to those described for the abdominal masses (cases 2, 5, 7 and 9).

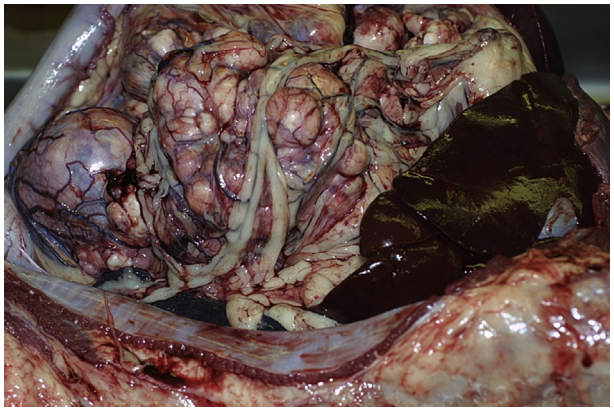

Fig. 1. Abdominal cavity in case 7 showing a large multilobulated mass that encompasses the mesentery, small intestine, kidneys and diaphragm.

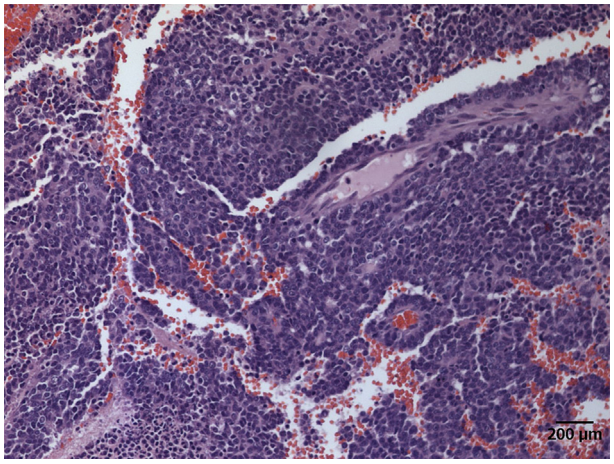

Fig. 2. Abdominal mass in case 1. The densely cellular neoplasm is composed of sheets of round to oval cells with distinct cell borders, scant lightly eosinophilic cytoplasm and round hyperchromatic nuclei. Note the pseudorosettes, in which the neoplastic cells surround blood vessels. HE. Bar, 200 µm.

Immunohistochemically, neoplastic cells from all cases were positive for synaptophysn (Fig. 4), NSE (Fig. 5) and chromogranin A (Fig. 6) and in one case (case 7) a strong immunoreactivity to tyrosine hydroxylase was present. All cases were negative for vimentin, cytokeratin, CD3 and CD79a (data not shown). Based on morphological and immunohistochemical features these cases were diagnosed as peripheral neuroblastomas and in cases 2, 5, 7 and 9

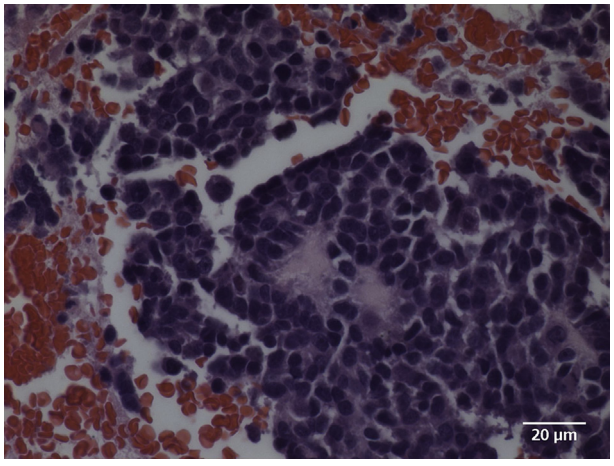

Fig. 3. Abdominal mass in case 2. Occasional Homer-Wright rosettes are evident, characterized by central granular to fibrillar eosinophilic material surrounded by rows of neoplastic cells. HE. Bar, 20 µm.

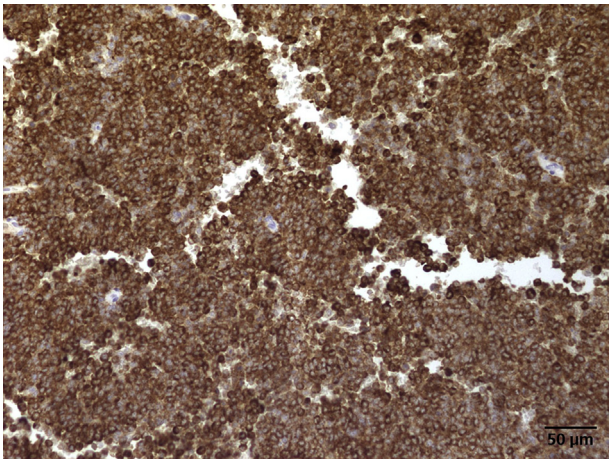

Fig. 4. Abdominal mass in case 5. A large number of neoplastic cells show strong positive labelling for synaptophysin. IHC. Bar, 50 µm.

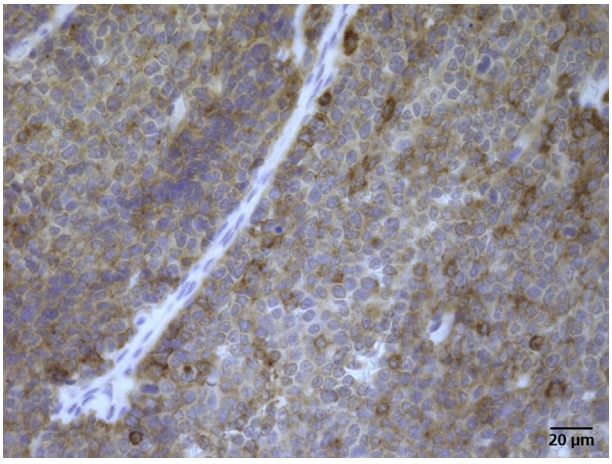

Fig. 6. Abdominal mass in case 5. A large number of neoplastic cells show strong positive cytoplasmic labelling for chromogranin A. IHC. Bar, 20 µm.

the diagnosis of metastatic peripheral neuroblastoma was made.

Discussion

PNTs including neuroblastomas are derived from the progenitor cells of the sympathetic nervous system that migrate from the neural crest to form the sympathetic ganglia, the chromaffin cells of the adrenal medulla and the paraganglia, thus reflecting the typical localization of these tumours (Park *et al.*, 2008). The mechanism that causes persistence of embryonal cells that later give rise to PNTs is unknown in dogs and it is only partially understood in man (Jiang *et al.*, 2011). In children, alterations in embryonic genes controlling neural crest development are likely to underlie the altered proliferation and differentiation of neuroblastomas. Recent investigations have suggested that mutations in the *Phox2B* genes, which control part of the sympathetic nervous system are involved (van Limpt *et al.*, 2005).

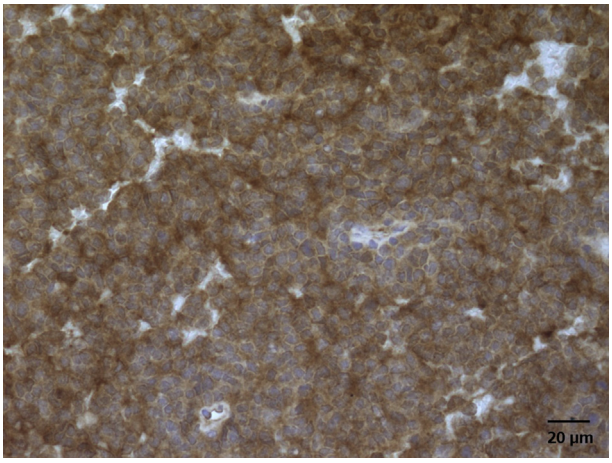

Fig. 5. Abdominal mass in case 3. A large number of neoplastic cells show moderate to strong positive labelling for NSE. IHC. Bar, 20 µm.

Clinical presentation of peripheral neuroblastoma is highly variable in man (Park *et al.*, 2008). This also appears to be the case in the dog where clinical presentation was dependent on the site of tumour origin and disease extent. Lethargy and anorexia were the most common non-specific presenting signs. Patients who had localized or disseminated disease were diagnosed after testing for unassociated medical conditions and the most common finding was the large size of the abdominal masses. In human cases, paraneoplastic syndromes often aid in the diagnosis of the disease and have been associated with episodes of secretory diarrhoea (Kerner–Morrison syndrome) due to tumour secretion of vasointestinal peptides. In the present study, three of the nine cases had diarrhoea, but whether there was tumour secretion of vasointestinal peptides is unknown. A series of biological factors have been shown to predict the clinical behaviour of neuroblastoma in children, including, but not limited to, the presence of genetic mutations such as the MYCN amplification and neurotrophin signalling alterations (Park *et al.*, 2008). Based on these cases and the previously published reports, it is highly likely that canine peripheral neuroblastomas are malignant, although the mechanism involved in tumourigenesis is unknown. Interestingly, Labrador retrievers and boxers appear to be overrepresented (44 and 22%, respectively) suggesting that a hereditary component is involved. No age or sex predisposition was evident and the majority of the cases occurred in animals under 2 years of age. Interestingly, age is an important clinical prognostic factor in children (Park *et al.*, 2008). Patients older than

1–2 years have a worse prognosis that those who are younger and this may also be the case in dogs, but the lack of specific clinical signs prevents early detection of canine tumours and thus a prognosis for early and late onset of disease. Human peripheral neuroblastomas can disseminate either through the lymphatics or by the haematogenous route, with the bone, bone marrow and liver being the most common sites of haematogenous spread (Matsushima *et al.*, 1998). In two of the present cases where a full necropsy examination was performed, metastasis to the liver was confirmed, suggesting a similar behaviour in dogs.

This report presents the largest published case series of canine peripheral neuroblastomas. Based on these observations and the previous publications there may be a predisposition for the Labrador retriever and boxer breeds, although a larger case series is needed for further evaluation (Louden *et al.*, 1992). Because the majority of dogs were either humanely destroyed at the time of diagnosis or no further follow-up was available, it was difficult to assess any correlation between treatment and prognosis. Metastatic disease was identified in four of nine dogs. This suggests that the biological behaviour of canine peripheral neuroblastomas mimics that of children with a late detection, by having a similar route of dissemination, although a larger case series would also be needed to confirm this observation. Hopefully, this report will result in an increased recognition of this tumour in dogs and lead to a better understanding of its biological behaviour and histogenesis.

Conflict of Interest Statement

No conflict of interest is disclosed by the authors of this paper.

References

Capucchio MT, Lotti D, Cornaglia E, Valenza F, Schiffer D (2003) Histological and immunohistochemical study of a neuroblastoma in a dog. *Clinical Neuropathology*, **22**, 176–179.

Forrest LJ, Galbreath EJ, Dubielzig RR, MacEwen EG (1997) Peripheral neuroblastoma in a dog. *Veterinary Radiology and Ultrasound*, **38**, 457–460.

Frye FL, Clement ED (1970) Sympathicoblastoma in a dog. *Journal of the American Veterinary Medical Association*, **156**, 900–901.

Jiang M, Stanke J, Lahti JM (2011) The connections between neural crest development and neuroblastoma. *Current Topics in Developmental Biology*, **94**, 77–127.

Koestner A, Bilzer T, Fatzer R, Schulman FY, Summers BA *et al.* (1999) Histological classification of the tumors of the nervous system of domestic animals. In: *World Health Organization, Histological Classification of Tumors of Domestic Animals*, 2nd Edit., Vol. 5, FY Schulman, Ed., The Armed Forces Institute of Pathology, Washington DC, pp. 26–27.

Louden C, Patterson JS, Sandusky GE (1992) Peripheral neuroblastomas in two dogs. *Journal of Veterinary Diagnostic Investigation*, **4**, 476–480.

Marcotte L, McConkey SE, Hanna P, Foley P, Burton S (2004) Malignant adrenal neuroblastoma in a young dog. *Canadian Veterinary Journal*, **45**, 773–776.

Matsushima S, Maruyama T, Torii M (1998) Peripheral neuroblastoma in a young beagle dog. *Toxicologic Pathology*, **26**, 806–809.

Park JR, Eggert A, Caron H (2008) Neuroblastoma: biology, prognosis, and treatment. *Pediatric Clinics of North America*, **55**, 97–120.

Steinberg H, Peek SF, Nelson KM (2006) Neuroblastoma with neuronal differentiation in the spinal cord in an Aberdeen Angus heifer calf. *Veterinary Pathology*, **43**, 193–197.

Suzuki M, Uchida K, Taniguchi K, Yamaguchi R, Tateyama S (2003) Peripheral neuroblastoma in a young Labrador retriever. *Journal of Veterinary Medical Science*, **65**, 271–274.

Uchida K, Murakami T, Tometsuka T, Iwakiri A, Yamaguchi R *et al.* (1998) Peripheral neuroblastoma and primitive neuroectodermal tumor in Japanese black cattle. *Journal of Veterinary Medical Science*, **60**, 871–875.

van Limpt V, Chan A, Schramm A, Eggert A, Versteeg R (2005) *Phox2B* mutations and the delta-notch pathway in neuroblastoma. *Cancer Letters*, **228**, 59–63.

[*Received, August 22nd, 2013*
Accepted, November 27th, 2013]