A true “triphasic” pattern: thoracolumbar spinal tumor in a young dog

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Abstract: An 8-month-old male Bernese Mountain Dog was referred with a history of hindlimb weakness that progressed to paresis on the right side. An intradural mass was detected in the spinal canal at the level of the 2nd and 3rd lumbar vertebrae. During surgical removal, 2 small fragments of the mass were prepared for cytologic examination by the squash technique. Cytologic examination revealed 3 different cell types: mesenchymal (stromal) cells, epithelial cells, and small undifferentiated hyperchromatic cells. On the basis of location and the triphasic cytologic pattern, a diagnosis of spinal nephroblastoma (thoracolumbar spinal tumor of young dogs) was made; histologic examination of the mass confirmed the cytologic diagnosis. To our knowledge, this is the first report of a triphasic pattern in a cytologic sample; recognizing this pattern is an important aid in reaching a definitive cytologic diagnosis. (Vet Clin Pathol. 2007;36:00-00)

Key Words: Cytology, dog, nephroblastoma, spinal mass, spinal tumor, thoracolumbar

An 8-month-old male Bernese Mountain Dog was referred for neurologic evaluation with a history of hindlimb weakness that progressed to paresis on the right side. On physical examination, the dog was found to be in good general condition. Neurologic examination revealed severe right hindlimb paresis with loss of conscious proprioception. The patellar and flexor reflexes were normal. The left hindlimb was normal. A lesion in the thoracolumbar segments (T3–L3) of the spinal cord was suspected, and the dog was admitted for further investigation. Magnetic resonance imaging examination was carried out using a low-field magnet (ESAOTE VET-MR, Genoa, Italy). Spin-echo T1- and T2-weighted images were obtained in the transverse, sagittal, and dorsal planes. Contrast-enhanced T1-weighted images were also obtained after an intravenous bolus (0.1 mmol/kg) of gadopentetate dimeglumine (Magnevist, Schering, Berlin, Germany). A well-demarcated, ovoid, space-occupying lesion was detected in the spinal canal at the level of the 2nd and 3rd lumbar vertebrae. The mass appeared to be within the spinal cord, and there was sufficient and homogeneous contrast enhancement. On transverse and dorsal images, the mass appeared to be mainly on the right side, occupying most of the cross-sectional area of the spinal cord. The mass was surgically removed via a right-sided hemilaminectomy. Opening of the dura mater confirmed that the mass was extradural and extramedullary. It was fully removed by blunt dissection. The dog’s recovery from surgery was uneventful and its neurologic condition improved in the following 2 weeks. By 4 months after surgery, the dog was in good general condition, but there was still mild paresis of the right hindlimb.

Each of 2 small fragments of the mass was crushed between 2 glass slides to prepare smears for cytologic examination. The resulting 4 slides were air-dried and then stained in an automatic slide stainer (7100 Aerospray Slide Stainer, Wescor Inc, Logan, UT, USA) with May–Grunwald–Giemsa. Microscopic examination revealed the smears to be highly cellular and of adequate quality. Each consisted of a mixture of 3 different cellular types: mesenchymal or stromal cells, epithelial cells, and undifferentiated small hyperchromatic cells.

The mesenchymal component predominated. The spindle-shaped cells were organized in loose to moderately aggregated clusters in an irregular storiform architectural arrangement. Cells were pleomorphic, ranging from fibroblastic cells with oval nuclei to well-differentiated smooth muscle-like cells with elongated, cigar-shaped nuclei, finely stippled chromatin and 1 to many small nucleoli surrounded by a small amount of fine, basophilic cytoplasm, sometimes with indistinct borders. In some fields, the stromal cells were intermixed with small undifferentiated cells and epithelial cells (Figure 1). There was occasionally a small amount of myxoid eosinophilic matrix surrounding the clusters of mesenchymal cells.

The epithelial cells were mainly organized in cohesive 3-dimensional structures resembling papillae, acini (Figure 2), and glomeruli (Figure 3), as well as in pseudorosettes. The cells were moderately pleomorphic; some were round with central nuclei and high N:C ratios, while others, mainly at the periphery of the clusters, were cuboidal or columnar and had more abundant basophilic cytoplasm. The latter cells were 2–2.5 times the diameter of an erythrocyte. The nuclei had finely stippled chromatin and 0–3 pale nucleoli. A few normal mitotic figures (<1 per high power field [HPF]) were observed.

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The last cellular element consisted of undifferentiated round, oval, or slightly spindle-shaped cells, 1.5 times the diameter of an erythrocyte. They were organized in loose to dense aggregates in which variable nuclear molding was observed (Figure 3), irregular sheets or cords, or occasionally, structures resembling glomeruli (Figure 4). Nuclei were small, ovoid, and dark blue, with inconspicuous nucleoli, and very little or no apparent cytoplasm. These cells were interpreted as immature, undifferentiated blastema cells. On the basis of location and cytologic features, the mass was diagnosed as a spinal nephroblastoma (thoracolumbar spinal tumor of young dogs).

For histologic examination, the tissue was fixed in 10% neutral buffered formalin and embedded in paraffin wax. Sections of 5 μm were stained with H&E and periodic acid-Schiff. Primary antibodies to glial fibrillary acidic protein (GFAP) (polyclonal, 1:500; Dako, Milan, Italy), vimentin (1:200; Dako), and pancytokeratin (MNF 116 clone, 1:400; Dako) were applied by use of the streptavidin-biotin-peroxidase complex and diaminobenzidine, with counterstaining by Carazzi’s hematoxylin.

Histologic examination of the tumor revealed both solid and glandular patterns of growth. The solid areas were characterized by a dense population of poorly differentiated spindle-shaped cells with ovoid nuclei and small amounts of eosinophilic cytoplasm organized in sheets and fascicles. The glandular areas consisted of tubules and acinar structures lined by columnar to pseudostratified epithelium, resting on a distinct basal lamina. Occasional structures resembling embryonal glomeruli (Figure 5) or pseudorosettes were

Figure 1. A cohesive 3-dimensional cluster of pleomorphic spindle-shaped stromal cells (white arrow) intermixed with epithelial cells (arrowhead) and smaller, pyknotic, blastemal cells (black arrow). May-Grünwald-Giemsa, ×60 objective.

Figure 2. An epithelial cell cluster showing acinar arrangements. May-Grünwald-Giemsa, ×100 objective.

Figure 3. Large pleomorphic epithelial cells forming a glomeruloid structure (black arrow) and smaller hyperchromic blastema cells organized in dense, cohesive aggregates (white arrow). May-Grünwald-Giemsa, ×100 objective.

Figure 4. A cohesive glomerular structure composed of hyperchromatic undifferentiated blastema cells. May-Grünwald-Giemsa, ×100 objective.

Figure 5. A cohesive glomerular structure composed of hyperchromatic undifferentiated blastema cells. May-Grünwald-Giemsa, ×100 objective.
observed. A delicate fibrous stroma supported the solid and
glandular areas and also supported nodular aggregates of
small and round blastemal cells with scant cytoplasm,
hyperchromatic nuclei with coarse chromatin, and inconspic-
uous small nucleoli. Mitotic figures were numerous (15 per
HPF). Spindle-shaped cells and blastemal cells stained
positively for vimentin, and most of the epithelial structures
were positive for pancytokeratin (Figure 6). Some of
the stromal cells were GFAP-positive, as were nerve fibers
trapped in the tumor. The final diagnosis was thoracolumbar
spinal cord tumor (spinal nephroblastoma).

Discussion

It is generally possible to identify the histogenesis of a tumor
from the architectural arrangement of the cells (eg, acinar or
papillary architecture is typical of epithelial neoplasms, while
storiform or stromal architecture indicates mesenchymal
origin), but in rare cases, the simultaneous occurrence of 2
distinct populations of neoplastic cells is observed. This
so-called “biphasic pattern” is well known in human cyto-
pathology and has also been described in veterinary cyto-
pathology.2,3 Much less common is the so-called “triphasic
pattern,” denoting the coexistence in the same smear of 3
different tumor cell populations. Although extensively
described both histologically and cytologically for human
nephroblastoma, it has not, to our knowledge, been
described in cytologic specimens of animal tumors.

Nephroblastoma (also reported as embryonal nephroma,
embryonal adenosarcoma, renal adenosarcoma, Wilms’ tu-
mor, and thoracolumbar spinal tumor of young dogs) is one of
the most important human pediatric neoplasms and accounts
for 6% of all pediatric cancers.1 The term “blastoma” defines
the malignant counterpart of embryonic, rather than mature
tissue, and microscopically, nephroblastoma recapitulates the
embryologic development of the kidney. To our knowledge,
this is the first cytologic description of the blastemal com-
ponent in a tumor from a dog. Confusion can occur in dif-
derentiating epithelial from blastemal cells. The distinctive
aspects of the blastemal component, when compared to the
epithelial component, are cell size (epithelial cells are 2–2.5
times the diameter of an erythrocyte and blastemal cells are 1.5
times the size of an erythrocyte), higher N:C ratio, inconspic-
uous nucleoli (2–3 nucleoli are visible in epithelial cells), and
scanty, hyperchromatic, basophilic cytoplasm (Figure 3).

In the case described here, the histologic features of
differentiated spindle cells, together with tubular and
glomerular structures, were consistent with ectopic primordial
renal tissue, suggesting that the tumor was a spinal nephro-
blastoma. This hypothesis was strongly supported by the
immunohistochemical findings. Canine renal nephroblastoma
demonstrates distinct cytokeratin and vimentin immunoreac-
tivity patterns that reflect the composition of this specific
neoplasm. Mesenchymal and blastemal cells show positive
immunoreactivity for vimentin, while the epithelial compo-
nent shows positive immunoreactivity for cytokeratin. In
addition, spinal nephroblastoma lacks immunoreactivity for
GFAP.

Spinal nephroblastoma must be differentiated histologi-
cally from some other neoplasms, most importantly, ependy-
momas. In a previous article, authors7 used a battery of
immunohistochemical stains to differentiate spinal nephro-
blastoma and ependymomas; spinal cord nephroblastomas
were consistently positive for cytokeratin, while ependymo-
mas were consistently negative. Positive cytokeratin immu-
noreactivity also rules out the differential diagnoses of
primitive neuroectodermal tumor or poorly differentiated
astrocytoma.

Thoracolumbar spinal tumor of young, large-breed dogs
is an uncommon intradural, extramedullary neoplasm, usu-
ally located between the 10th thoracic and 2nd lumbar
vertebrae,8,9 and has considerable histologic similarity to
renal nephroblastoma. The histogenesis of this tumor has
not been firmly established, although it could well arise from
remnants of renal primordium, which becomes trapped

Figure 5. Histologic section of the tumor. Tubular patterns are mixed
with solid areas of poorly differentiated cells, among which glomerular
structures are recognizable (arrow). H&E, ×10 objective.

Figure 6. Histologic section of the tumor. Positive staining for cytokeratin
can be seen in some of the epithelial cells forming tubular structures. ABC
immunohistochemical stain, Carazzi’s hematoxylin counterstain, ×20
objective.
between the dura and the developing spinal cord. While the histomorphology and location are suggestive of ectopic nephroblastoma—and one of these tumors was reported to stain positively for Wilms’ Tumor Gene Product WT1—the name recommended by the World Health Organization Histological Classification of Tumors of Domestic Animals is “thoracolumbar spinal cord tumor of young dogs.”

Neel and Dean gave a cytologic description of this tumor. Both epithelial and stromal components showed considerable resemblance to those described in the present case, but the embryonic neoplastic tissue and the coexistence of 3 different tumor cell populations were not described. This might be because they used a touch imprint technique to prepare slides for cytologic examination. The squash or crush technique, which we used, provides information about both cytologic and architectural features of lesions of the nervous system and has been shown to be of greater value in the diagnosis of central nervous system lesions in dogs and cats, with fewer nondiagnostic specimens than those obtained by touch imprints.

References