Managing Epiglottal Chondrosarcoma of a Dog: A Case Report

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ABSTRACT
Primary chondrosarcoma was found in the epiglottis of a 6-year-old, neutered, male Boxer Cross. Clinically, there was upper respiratory noise, and a 3.2 x 2.8 x 2.7 cm ovoid mass involving the epiglottis was observed. No abnormalities were detected on radiographic examinations (X-ray) of the chest or elsewhere. Grossly, the excised mass was firm to hard and had a lobular pattern of cartilage with a translucent grayish white color on the cut surface. Histologically, the epiglottal submucosa had a non-encapsulated fairly demarcated multinodular neoplasm composed of streams of cells admixed with moderate to abundant amounts of chondroid substance. The neoplastic cells were positive for vimentin and S-100 protein, and negative for pan-cytokeratin. Histochemically, the matrix was deeply stained for Alcian Blue (pH 2.5)-Periodic Acid Schiff (PAS). To the best of our knowledge, this is the first report of extraskeletal chondrosarcoma arising primarily in the epiglottis of a dog with immunohistochemical and histochemical characterization.

INTRODUCTION
Primary neoplasms of larynx are rare in the dog and cat.1-5 In dogs, the reported laryngeal neoplasms include papilloma, squamous cell carcinoma, rhabdomyoma, rhabdomyosarcoma, oncocytoma, chondroma, chondrosarcoma, lipoma, leiomyoma, and leiomyosarcoma.1-5 Chondrosarcoma is a malignant tumor of cartilage; it may be primary, originating inside a bone (central) or from the periosteum (peri peripheral), or secondary, initiating through malignant change in osteochondromas.6-7 Microscopically, the malignant cells produce characteristic chondroid and fibrous matrix. Macroscopically, the chondrosarcoma develops on the bone surface, as large masses ranging in size from 2 cm to 20 cm in diameter with lobulated surfaces, firm or hard consistency, and relatively well bordered. Chondrosarcoma in dogs has a significantly higher incidence compared to osteosarcoma, where it frequently is located in flat bones.6-7 The incidence of chondrosarcoma has been often reported in adult animals. The tumor has been particularly studied in dogs, where it...
Fig 1A: Oral cavity; dog. An ovoid, epiglottic mass lodged against posterior wall of pharynx.

Fig. 1B. Epiglottal mass; dog. An expansile tumor (T) arising subjacent to the mucosa (arrow) which compresses and focally infiltrates the adjacent adipose tissue (asterisk). HE stain. Bar = 250 μm.

Fig. 1C. Epiglottal mass; dog. A higher magnification of fig 2 shows well-differentiated chondroid area where neoplastic cells are located within lacunae surrounded by elastic cartilage matrix. HE stain. Bar = 50 μm.

Fig. 1D. Epiglottal mass; dog. Cartilage matrix of the chondroid lobules (T) stained deeply with Alcian blue-PAS with negative staining of the adjacent adipose tissue (asterisk). Bar = 150 μm.

Fig. 1E. Epiglottal mass; dog. Immunohistochemistry for vimentin. The neoplastic cells within chondroid lobules in the mass (arrow heads) and bordering adipose tissue (asterisks) are strongly immunolabeled. The overlying mucosa (arrow) show negative staining for vimentin. Bar = 250 μm.

Fig. 1F. Epiglottal mass; dog. Immunohistochemistry for S-100. The neoplastic cells within chondroid lobules in the mass are strongly immunolabeled. Bar = 250 μm.
account for about 10% of all bone sarcomas, predominantly affecting large breed dogs especially the Boxer and German Shepherd breeds, with no sex preference. Chondrosarcomas have less tendency to metastasize compared with osteosarcomas. Metastasis of chondrosarcomas occurs via the hematogenous route mainly to the lung. Typically, this occurs later in the course of the tumor development, but only in approximately 10% of the cases.

The only previously reported cases of chondrosarcoma of the larynx in veterinary literatures included a 10-year-old male Boxer and an 8-year-old male Doberman pinscher where the tumors were attached to the left arytenoid cartilage but were not evaluated immunohistochemically. There were no reports of chondrosarcoma of the epiglottis within veterinary literature. In this report, we present a low grade chondrosarcoma arising from the epiglottis of a dog which was three treated with surgical excision. In addition; gross, histologic, immunohistochemical, and histochemical findings of this tumor are discussed.

Case Report

A 6-year-old, neutered, male Boxer Cross was presented with a primary complaint of gagging when eating for a time period of approximately one month. The owner also reported a change in bark, changing from a sharp tone to dull. Upon clinical examination, there was upper respiratory tract noise, and an ovoid lump involving the epiglottis was observed (Fig 1A). No abnormalities were detected on radiographic examinations (X-ray) of the chest or elsewhere. No significant abnormalities were seen in any blood samples from this animal as hematology, serum chemistry, and liver function tests were within normal limits.

The animal was sedated with acepromazine maleate (0.03mg/kg; ACP injection; Novartis Animal Health) and buprenorphine (0.03ml/kg; Vetergesic 0.3mg/ml; Alstoe Ltd.) and induced with propofol (0.4ml/kg; Propoflo; Abbott Animal Health). Simple interrupted stay sutures of 2-0 polydioxanone suture (PDS) were placed through the epiglottis.

The mass appeared attached caudally to the epiglottis by a pedicle and was ligated with polyglactin 910 (vicryl; Ethicon). The epiglottis was sectioned caudal to the mass and the mass was totally excised. The bleeding was very minimal and was stopped with gentle pressure. The Stay sutures were then removed. The animal also was given METACAM Solution for subcutaneous (Sc) Injection, Betamox LA 150mg/ml Suspension for Injection (Amoxicillin Trihydrate 172.1 mg/ml) and Ketamine during recovery. Routine post operative treatment consisted of Metacam Oral Suspension and Nisamox Tablets 250mg (1 tablet twice a day) for 2 weeks. Adjuvant chemotherapy was not recommended for this animal. Post operative checks were routinely performed at one day post surgery then 1 week, 1 month, 3 months, 6 months and a year post surgery then in general at 6 month intervals after this.

Grossly, the excised mass measured 3.2 x 2.8 x 2.7 cm, was firm to hard, and had a lobular pattern of cartilage with its translucent grayish white color on cut surface. The removed epiglottal mass was fixed in 10% buffered formalin, embedded in paraffin wax, and sectioned at thickness of 4 μm. To better characterize the neoplasm, deparaffinized sections were stained histochemically with hematoxylin and eosin (HE), Alcian Blue (pH 2.5)-Periodic Acid Schiff (PAS), and Von Gieson’s stain. In addition, immunohistochemistry for vimentin and S-100 protein, and pan-cytokeratin were performed.

The detection of different antigens to further differentiate the immunophenotype of the tumor was performed using avidin-biotin peroxidase complex (ABC) technique using three antibodies including: a monoclonal mouse antibody against Vimentina (1:200, DAKO), S-100 proteinb, and polyclonal pancytokeratin (1:100, DAKO)8-10 For immunohistochemical studies, additional sections from the tumor were deparaf-
finized using a graduated xylene and alcohol solutions, then rehydrated in distilled water using phosphate buffered saline (PBS), pH 7.4. Sections were incubated in a 3% hydrogen peroxide solution. Antigen retrieval was done using Citra solution d (BioGenex), and heating in a microwave oven for 10-minutes on a power setting of 600 watts. Slides were allowed to cool for 20 minutes and rinsed in a several changes of distilled water. Slides were incubated for 2-hours with one of the three primary antibodies, and then washed in PBS for 5-minutes, and then incubated for twenty minutes with the secondary antibody. The slides were rinsed with buffer, incubated with the label (prediluted horseradish peroxidase113 labeled streptavidin in PBS with carrier protein and 0.01% thimerosal) for 20-minutes at room temperature, rinsed well with buffer, and DAB e 114 (BioGenex) was applied to the sections for 5- minutes at room temperature. The color change was monitored on positive control slides and stopped by immersing all slides in deionized water. Contrast staining was performed using six Mayer's hematoxylin. Slides were dehydrated through ascending concentrations of alcohol solution and xylene, and were then coverslipped. Positive control tissue and negative reagent control were run simultaneously for verification of immunohistochemical staining results.

**RESULTS**

Microscopically, the epiglottal mass was rimmed by an outer stratified squamous non-keratinized epithelium, and the subjacent submucosa was markedly expanded by a non-encapsulated fairly demarcated multilobular neoplasm (Fig. 1B). The neoplasm was composed of streams of cells admixed with moderate to abundant amounts of a pale amphophilic to eosinophilic matrix (chondroid). The neoplastic cells were spindle, stellate or polygonal in shape with indistinct cellular borders and little to moderate eosinophilic lacy to multiloculated cytoplasm (Fig. 1C). Nuclei were ovoid with finely stippled chromatin and 1 small magenta nucleolus. Anisocytosis and anisokaryosis were moderate to marked with some karyomegaly and binucleated and multinucleated cells. Mitotic figures were uncommon with only 1 present in 10 random high power fields (40x). Scattered areas of edema, hemorrhage, and fat necrosis were present in the adjacent epiglottal tissue.

A diagnosis of low grade (grade I) chondrosarcoma was made based on the light microscopic examination. This tumor appeared to be completely excised but with thin excisional margins (< 3.0 mm in thickness in some areas). In addition, it apparently had involved the lingual surface and the apical portion of the laryngeal surface, as indicated by the presence stratified squamous non-keratinized epithelium with no evidence of respiratory epithelium in any of the sections examined. Immunohistochemical examination of the tumor, to further elucidate histogenesis, demonstrated strong cytoplasmic immunoreactivity to vimentin (Fig. 1E), confirming their mesenchymal origin. The neoplastic cells showed moderate to marked positive cytoplasmic staining to S-100 (Fig. 1F). Conversely, no neoplastic cells showed immunoreactivity for pan161 cytokeratin (for epithelial origin). For all antibodies, significant immunoreactivity was present in eight positive control sections, but absent in the negative controls, confirming validity of the staining technique.

Moreover, the intercellular matrix was histochemically stained dark blue with Alcian blue-PAS (Fig 1D), which often isolates cells into individual lacunae. Van Gieson staining showed that the mass contained elastic fibers consistent with the presence of elastic cartilaginous tissue. Based on the histologic findings (cartilaginous differentiation of neoplastic cells and matrix), and histochemical and immunohistochemical examination, a low-grade epiglottal chondrosarcoma was definitely diagnosed.

Following the surgery, routine follow up indicated that the animal appeared normal. There was no swelling or discomfort at the surgery site, nor any evidence of recurrence. Approximately 2- years following the
tumor’s resection, radiographic examination revealed an abdominal mass present in the liver; however, the owner declined further treatment, and the animal was euthanized.

**DISCUSSION**

Laryngeal tumors are extremely rare in small animal veterinary medicine; they include epithelial and mesenchymal types. Previous studies have reported that squamous cell carcinoma and rhabdomyoma are the most commonly encountered types of canine laryngeal neoplasia. Although two cases of laryngeal chondrosarcomas have been reported from a ten-year-old Boxer and an eight-year-old Doberman pinscher, in these tumors they were attached to the left arytenoids. Therefore, no report of epiglottis chondrosarcoma has been reported in veterinary literature.

Histologically, the larynx cartilages include thyroid cartilage, cricoid cartilage, arytenoid cartilages, corniculate cartilages and cuneiform cartilages; all are composed of hyaline cartilage which is the most abundant type of cartilage in the body. The epiglottis is composed of elastic cartilage. All cartilage is composed of chondrocytes located in lacunae and avascular extracellular matrix. The chondrocytes often exist in small aggregates (isogenous clusters). Elastic cartilage also has elastic fibers in the matrix.

In human literature, cartilaginous lesions of the larynx are classified as either neoplastic or metaplastic in origin, chondroma and chondrosarcoma being neoplastic lesions and metaplastic cartilaginous nodules (chondrometaplasia) being metaplastic in origin. It is important to differentiate low-grade chondrosarcomas from chondromas. The symptoms are the same but chondromas are more likely to be asymptomatic. The site of origin is similar but chondromas are almost always less than 2 cm in size, whereas chondrosarcomas are larger than 3 cm. Microscopically, chondromas appear as normal cartilage with defined hypocellular lobules with no nuclear atypia and mitosis. Chondrometaplasia is a common lesion composed of well-defined nodules with metaplastic activity of chondrocytes, usually with no lobular pattern of chondrocytes or nuclear abnormalities. They must be differentiated from neoplastic cartilaginous lesions. The nodules are less than 1 cm usually on ventricular bands and vocal cords; and may be multifocal. Chondrosarcoma is a malignant tumor involving the cells that produce a cartilage matrix. Primary chondrosarcomas are uncommon in both dogs and humans, but have been occasionally reported. Most chondrosarcomas originate from the skeletal cartilage, whereas some occur in extraskeletal tissues that have preexisting cartilage tissue. In this report, the existing epiglottal mass in this dog was a low-grade chondrosarcoma, which was microscopically characterized by well-developed chondroid lobules, which was primarily originated from the elastic cartilage of the epiglottis. Histochemical staining and Immunohistochemical positivity for vimentin and S-100 protein, a standard marker for chondrogenic neoplasms, together with negativity for pan-cytokeratin confirmed the mesenchymal and chondroblastic origin. Liposarcoma could argued as a low but po-
tential\textsuperscript{11} differential diagnosis for this tumor, since both chondrosarcoma and liposarcoma are positive for S-100 protein and vimentin;\textsuperscript{8, 12} however, neoplastic chondrocytes in this case did not reveal clear lipid droplets in their cytoplasm.

Inadequate numbers of reports of cartilaginous tumors of the epiglottis in veterinary medical literature, and lack of archived case records and necropsy findings, pose some diagnostic and therapeutic challenges. There was no evidence of local or regional recurrence in this animal; however, an abdominal mass was observed by x-ray examination after over 2-years of the surgical excision of this epiglottal mass. As necropsy was not allowed, we remain uncertain about a histologic diagnosis of this abdominal mass.

We conclude that the epiglottal mass in this dog represented a low-grade chondrosarcoma, a rare neoplasm reported in dogs and humans, which was primarily originated from the epiglottis and was treated surgically. On the basis of the case in this report, the long-term prognosis of future cases of epiglottal chondrosarcomas should be considered guarded until more information has been gathered.

REFERENCES: