

## Cox-2 expression after chemotherapy in a canine nasal transitional cell carcinoma: a case report

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**ABSTRACT:** An eight year-old mixed breed dog was referred for evaluation with chronic sneezing, epistaxis and nasal bone deformation. A clinical exam revealed a deformity of the left nasal bone. Cytological evaluation of the nasal cavity showed round and cuboid cells in different stages of maturation. Computed tomography images identified a diffuse soft tissue mass in the nasal cavity. The histopathological diagnosis was transitional cell carcinoma. Chemotherapy with cisplatin and piroxicam was initiated. Computed tomography was used to follow the chemotherapy outcome. As many nasal carcinomas express COX-2, its expression was evaluated before and after chemotherapy. Computed tomography revealed a significant reduction of the tumour mass. Immunohistochemistry showed a marked decrease in COX-2 expression and after 1120 days, the dog remained without clinical signs. Thus, the use of piroxicam and cisplatin is revealed to be effective in the treatment of a nasal transitional carcinoma in a dog. Also, it is possible to postulate that a decrease in COX-2 expression after chemotherapy may be regarded as an indication for a good response to the treatment and favourable outcome. To the authors' knowledge this is the first report that evaluates COX-2 expression after chemotherapy.

**Keywords:** dog; cisplatin; piroxicam; nasal tumour

Primary tumours of the nasal cavity account for approximately 1% of all neoplasms that develop in dogs of middle age or older (Withrow 2007). Eighty percent of nasal tumours in dogs are malignant, and 60% to 75% are epithelial in origin (Wilson and Dungworth 2002). Nearly two-thirds of all nasal tumours in dogs are adenocarcinomas (Kondo et al. 2008) and are characterised by progressive, local invasion with bone destruction early in the disease (Saunders et al. 2003). The long-term prognosis for dogs with nasal carcinoma is poor. Patients usually die within six months of the diagnosis (Rassnick et al. 2006). Studies that have evaluated COX-2 expression in canine nasal tumours have found that 71% to 87% of nasal carcinomas are COX-2-positive (Borzacchiello et al. 2004; Kleiter et al. 2004; Impellizeri and Esplin 2008), suggesting that COX-2 may participate in nasal tumourigenesis. The enzyme seems to be involved in multiple events during tumour development and is upregulated early in oncogenesis. Considering the potentially critical role of COX-2 in epithelial tumour progression, inhibition

of COX-2 would seem to be a rational strategy to improve the outcome in canines (Kleiter et al. 2004).

### Case description

An eight year-old male mixed breed dog weighing 26 kg was referred with chronic epistaxis, chronic sneezing and snoring. A clinical exam revealed a mild deformity of the left nasal bone, and the dog was sensitive to palpation of this area. In addition, a deformity was observed on the left supraorbital area. The oral cavity exam was unremarkable. The submandibular lymph nodes were increased in size without elevated temperature or sensitivity. CBC and other biochemical parameters (urea, creatinine, ALT, GGT, SAP, albumin, glucose) were within their reference ranges. An intraoral dorsoventral radiograph of the nasal cavities revealed a loss of turbinate bone and nasal septum. Thorax radiographs with three views did not show any lung tissue masses. The dog was sedated (xylazine 0.5 mg/kg),

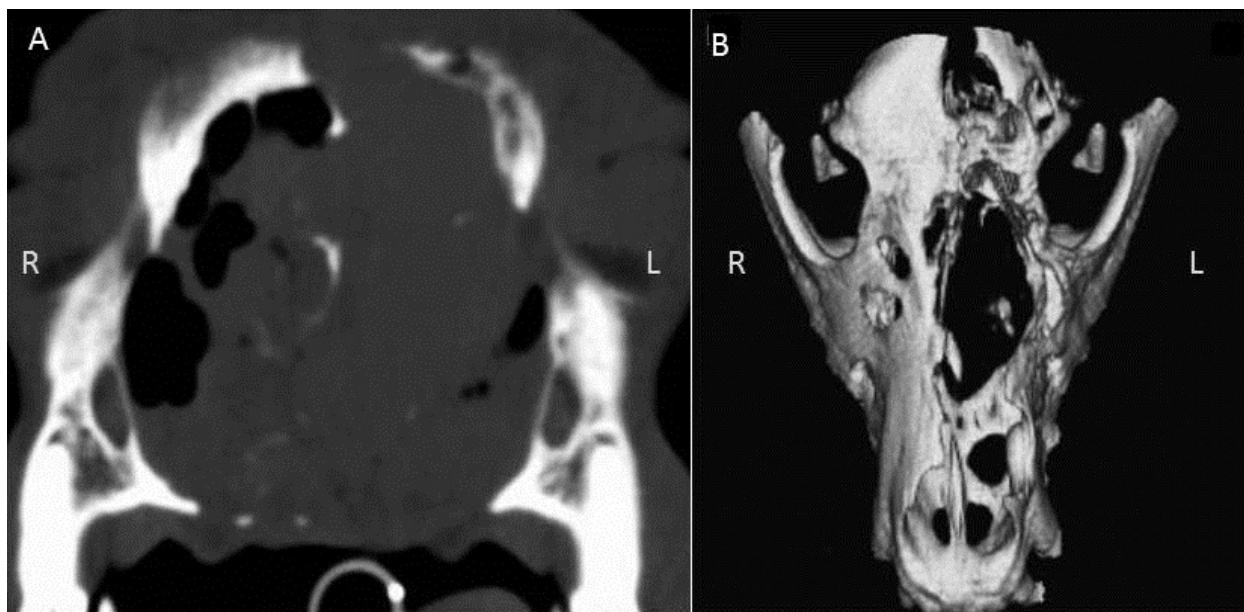


Figure 1. Tranverse CT image reveals a soft tissue density mass occupying most of the nasal cavity with lysis of the dorsal left nasal bone (A). Three-dimensional surface reconstruction CT scan showing lysis of the nasal bone, the nasal septum, the left maxillary sinus bone and the left frontal bone (B)

bilateral nasal cavity samples were taken with cotton swabs and fine needle aspirate was taken from the submandibular lymph nodes.

Cytological exam of the left nasal cavity showed numerous red blood cells and abundant round and cuboid cells in different stages of maturation. Vacuolar cytoplasm was observed in addition to anisokaryosis, central nuclei with prominent nucleoli and a variety of chromatin patterns. The right nasal cavity contained cells with similar features but not red blood cells. Neutrophil infiltration was observed bilaterally. Lymph node cytology was unremarkable. The possible diagnoses were adenocarcinoma, squamous cell carcinoma (SCC), transmissible venereal tumour (TVT) and nasal transitional cell carcinoma (TCC). To assist the diagnosis, computed tomography (CT) and a biopsy analysis were scheduled.

CT revealed a diffuse soft tissue mass located in the nasal cavity invading the frontal sinus. The mass identified in this case was composed of soft tissue and extended to two-thirds of both turbinates (Figure 1A). The nasal bone, the nasal septum, the left maxillary sinus bone and the left frontal bone all showed evidence of lysis (Figure 1B), but the cribriform plate and the ocular orbits remained intact. Using the criteria of the TNM staging system proposed by the World Health Organization (Owen 1980), the present case was defined as a bilateral

tumour with moderate bone destruction, bilateral retropharyngeal lymph node enlargement and no evidence of distant metastasis. According to the Theon system (Theon et al. 1993), we defined it as a bilateral neoplasm extending into the frontal sinus with erosion of the nasal bone and maxillary bone.

Transnostril core biopsies were obtained during rhinoscopy. For histological exams, the biopsies were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 4  $\mu$ m and stained with haematoxylin-eosin for evaluation by light microscopy. Microscope examination revealed a thick stratum with stratified cuboid cells among squamous and respiratory epithelia, vacuolated granular cytoplasm with prominent nuclei and clearly demarcated nucleoli. The cells were surrounded by a fibrovascular septum and necrotic areas. The diagnosis was, therefore, transitional cell carcinoma of the nasal epithelium. To evaluate COX-2 expression in this nasal carcinoma, biopsies were stained with COX-2 monoclonal antibodies with a repeat of the staining at the end of chemotherapy. For immunohistochemistry, sections (3  $\mu$ m) were mounted on silanised slides. A mouse monoclonal antibody raised against COX-2 (Clone 33; BD Transduction Lab, CA, USA) was used at 1 : 50 dilution. Immunohistochemistry was carried out using a two-step method (Novolink Polymer Detection System, Novacastra) according to the manufacturer's instructions. The sections were

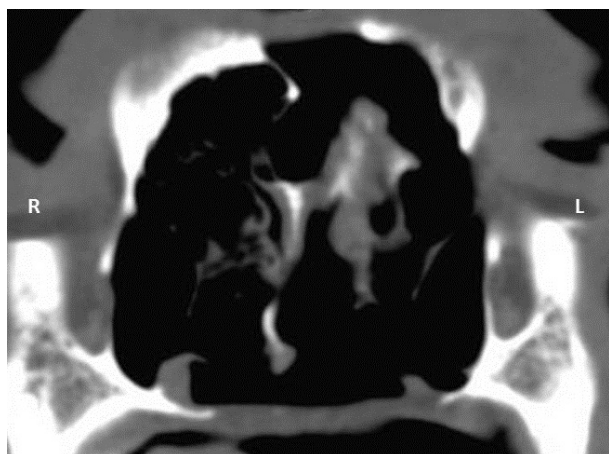


Figure 2. Transverse CT image reveals reduction of the neoplastic soft tissue after six sessions of chemotherapy

then developed in diaminobenzidine, counterstained with haematoxylin, dehydrated, cleared and mounted. Sections from normal canine kidney were used as controls. Immunohistochemical staining was evaluated according to a semi-quantitative scoring system based on the percentage of COX-2-positive cells (Belshaw et al. 2011), avoiding areas with inflammation and necrosis in both samples. The comparison of COX-2 intensity was determined in a blind study by a senior pathologist.

Chemotherapy was started with cisplatin (50 mg per m<sup>2</sup>) that was administered every 21 days for a total of six sessions. In addition, piroxicam (0.3mg/kg) was given every other day. Cisplatin was administered intravenously with diuresis over a period of six hours. The protocol used started with pre-hydration with NaCl 0.9 % (18 ml/kg/h) for four

hours. Then, cisplatin was diluted in NaCl 0.9% (7 ml/kg) and given over a 20-minute period. After cisplatin, Ranitidine (2 mg/kg) and metoclopramide (0.5 mg/kg) were administered intravenously to prevent vomiting. Finally, there was a two-hour postdiuresis period (18 ml/kg/h). Before each chemotherapy session, blood cell count, urea nitrogen, creatinine and phosphorus were measured to assess the side effects of the chemotherapy. The patient tolerated the chemotherapy without side effects. The epistaxis resolved after the first session, and the sneezing and deformity that were due to a small mass located in the maxillary and frontal bones disappeared after the second session.

At the end of the chemotherapy, a CT was performed and biopsies were taken again. The samples were immunostained with a COX-2 antibody and compared with the biopsies taken before treatment. The CT images showed the turbinates, and a clear reduction was observed in the amount of soft tissue mass. No neoplastic tissue invasion was observed in the nasal cavity or the frontal sinus (Figure 2). Further evaluations were performed at three month intervals. At the time of writing piroxicam is being administered indefinitely.

Before the treatment, COX-2 immunoreactivity was moderate in intensity, was found in < 50% of cells, and these were predominantly located in the cytoplasm (Figure 3A). COX-2 expression was weaker after the chemotherapy sessions and only < 1–9% of cells were positive (Figure 3B). Assessing COX-2 expression allowed us to confirm a favourable prognosis after chemotherapy. At the time of writing, the dog has survived for 1120 days without clinical signs.

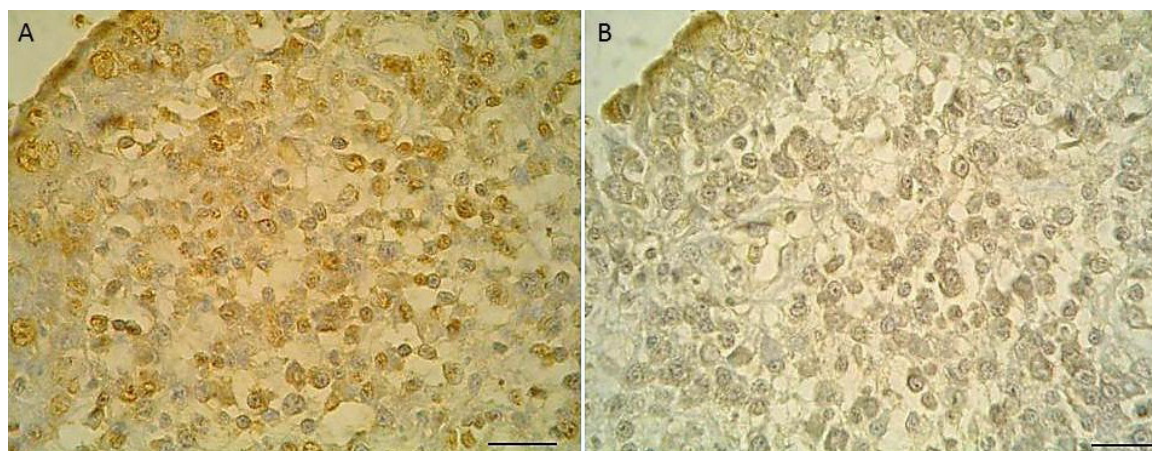


Figure 3. Immunohistochemistry of COX-2 expression in nasal neoplastic tissue. Moderate cytoplasmic immunostaining seen in < 50% of epithelial neoplastic cells (A). Mild and diffuse cytoplasmic immunostaining seen in < 9% of epithelial neoplastic cells (B). Bar 30 μm



## DISCUSSION AND CONCLUSIONS

In dogs, between 19% and 47% of chronic nasal diseases are neoplasias (Rassnick et al. 2002; Wilson and Dungworth 2002; Withrow 2007). Of all sinonasal tumors, approximately two thirds are of epithelial origin. The most frequent nasal neoplasias are adenocarcinomas, SCCs and TCCs (Saunders et al. 2003; Lana et al. 2004; Rassnick et al. 2006). Many studies and reports have described TCC in the nasal cavity and its prevalence among nasal neoplasias ranges from 12–50% (Theon et al. 1993; Borzacchiello et al. 2004; Langova et al. 2004; Impellizeri and Esplin 2008; Vanherberghen et al. 2009).

In this case, the CT was very important because it allowed the extent of the neoplasia to be determined and identified the affected bone structures. Thus, CT enhances the diagnosis of canine chronic nasal disease; it allows discrimination between neoplastic and non-neoplastic disease and identifies areas for rhinoscopy or biopsy analysis (Saunders et al. 2003). With CT images, it is possible to create a staging system that may be more accurate than that of the WHO because histologic grades are not useful in the determination of progression in patients with nasal tumours (Borzacchiello et al. 2004).

Based on the histopathology results and CT images in this case, RT could have been an appropriate treatment. RT has been shown to improve survival time in dogs with sinonasal tumours (Elliot and Mayer 2009). While surgery and chemotherapy have been used as sole treatment modalities for canine nasal tumours (Langova et al. 2004), RT used alone or in combination with surgery or chemotherapy can significantly improve patient survival time (Theon et al. 1993; Lana et al. 2004; Elliot and Mayer 2009). The availability of RT equipment is currently limited for many vet practitioners. Additionally, it may be declined by some owners due to the cost, acute side effects or the three- to four-week separation from their pet that may be required (Langova et al. 2004). In this case, the patient received chemotherapy with cisplatin and piroxicam. Both have been used for their antitumour activity in nasal neoplasias, such as melanoma and SCC (Boria et al. 2004). These compounds could act synergistically; apoptosis may be initiated by piroxicam, while the subsequent cell death may be due to cisplatin treatment (Boria et al. 2004).

Our results suggest that chemotherapy in canine nasal carcinomas may be a good choice when ra-

diotherapy is not possible. Some authors have not recommended the use of cisplatin with piroxicam, because their efficacy is minimal and is associated with gastrointestinal and renal toxicosis (Greene et al. 2007). Nevertheless, some authors reported antitumoural activity of both in bladder and nasal tumours with acceptable toxicity, although renal function was monitored carefully (Mohammed et al. 2003; Boria et al. 2004). The chemotherapy protocol described here was effective and well tolerated; it has kept the patient alive during the 1120 days since diagnosis. This individual outcome is similar to those achieved with radiotherapy (Elliot and Mayer 2009; Belshaw et al. 2011). The prognosis of dogs with untreated nasal carcinoma is poor, and the median survival time is 95 days. However, early control of the clinical signs during the first session of chemotherapy may help to increase the survival time (Rassnick et al. 2006). Epistaxis control in canine nasal neoplasia has been associated with longer survival time. In this case, the epistaxis was controlled after the second chemotherapy session. CT images allowed us to observe a decrease in the tumour mass that caused the epistaxis.

Many studies have demonstrated the expression of COX-2 in canine nasal carcinomas, melanomas and mammary tumours (Borzacchiello et al. 2004; Spugnini et al. 2005; Belshaw et al. 2011). It has been suggested that its possible role in canine tumorigenesis is related to its inhibition of apoptosis, promotion of cell proliferation, angiogenesis stimulation and dampening effects on immunity (Dore 2011). However, there is a great variability in the results of studies which have examined COX-2 expression in the same type of cancer and within a same species (Borzacchiello et al. 2004; Belshaw et al. 2011).

In this case, COX-2 expression was localised in the cytoplasm and had a granular appearance; however, it was also pericytoplasmic, as described in other reports (Borzacchiello et al. 2004; Impellizeri and Esplin 2008; Belshaw et al. 2011).

A marked decrease in COX-2 expression after chemotherapy allowed us to assess the patient without clinical signs. However, as COX-2 intensity and distribution is heterogeneous in neoplasia it cannot be used as a prognostic indicator (Dore 2011). Many studies and clinical reports have determined COX-2 expression in neoplastic biopsies with the aim of evaluating its prognostic relevance; however, the results have not been significant (Queiroga et al. 2010; Belshaw et al. 2011). Our report is the

first to evaluate COX-2 expression before and after chemotherapy. We observed a decrease in COX-2 expression after the chemotherapy. This was associated with a positive outcome related with a survival time of 1120 days without adverse clinical signs (information obtained from the owners during report preparation). A biopsy control during treatment (radiotherapy or chemotherapy) or at the end, followed by the determination of COX-2 expression, could facilitate the prediction of decreases in tumour mass. Nevertheless, many studies, in human and dogs, have yet to find any correlation between COX-2 and survival time. However, in all these mentioned studies there is no information about COX-2 expression after chemotherapy, because the investigators only tested clinical records or patient information (Queiroga et al. 2010; Belshaw et al. 2011).

The antineoplastic action of COX-2 inhibitors may not correlate with immunohistochemical COX-2 expression; rather, the effects on the tumour microenvironment and stromal cells may be more important (Belshaw et al. 2011).

In conclusion, the use of CT allows the observation of many anatomical features underlying epistaxis. The use of a chemotherapy protocol with cisplatin and piroxicam effectively controlled nasal TCC. This protocol could be used when radiotherapy is either inappropriate or not possible. We evaluated the effectiveness of the chemotherapy protocol by examining COX-2 expression after the chemotherapy and observed a decrease in COX-2 expression. This outcome was associated with a decrease in the tumour mass and long survival time.

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Received: 2013–07–11

Accepted after revision: 2013–11–22

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