Cervical swelling in a dog

Contributors

Ignacio Amarillo-Gómez[1,2], Trinidad Artacho Burgos[3], Jaume Alomar Huguet[4], María Cuvertoret-Sanz[4], Antonio Meléndez-Lazo[1]

[1] T-Cito

[2] Universitat Autònoma de Barcelona

[3] Hospital veterinario SOS Animal

[4] IDEXX Laboratories

Ignacio Amarillo-Gómez: ignacio.amarillogomez@gmail.com

Specimen

Fine needle aspirate biopsy of the right cervical area.

Signalment

10-year-old male hunting dog.

History

The dog presented for swelling of the right cranial cervical area.

Clinical findings

Initial clinical examination revealed cervical swelling and pain upon palpation of the right retropharyngeal lymph node area. The rest of the general physical examination, hematology and serum biochemistry did not reveal any alterations.

Fine needle aspiration of the cervical region was performed, and samples were submitted to the laboratory for cytologic examination (Figures 1-2).

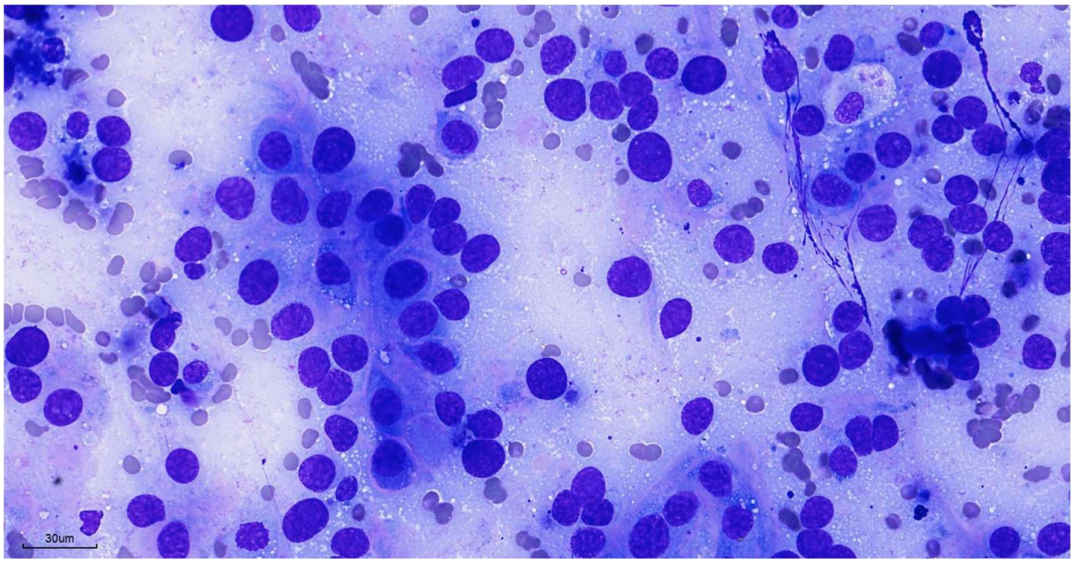

Figure 1. *Fine needle aspirate of the cervical region (Modified Wright Giemsa) (100x)*

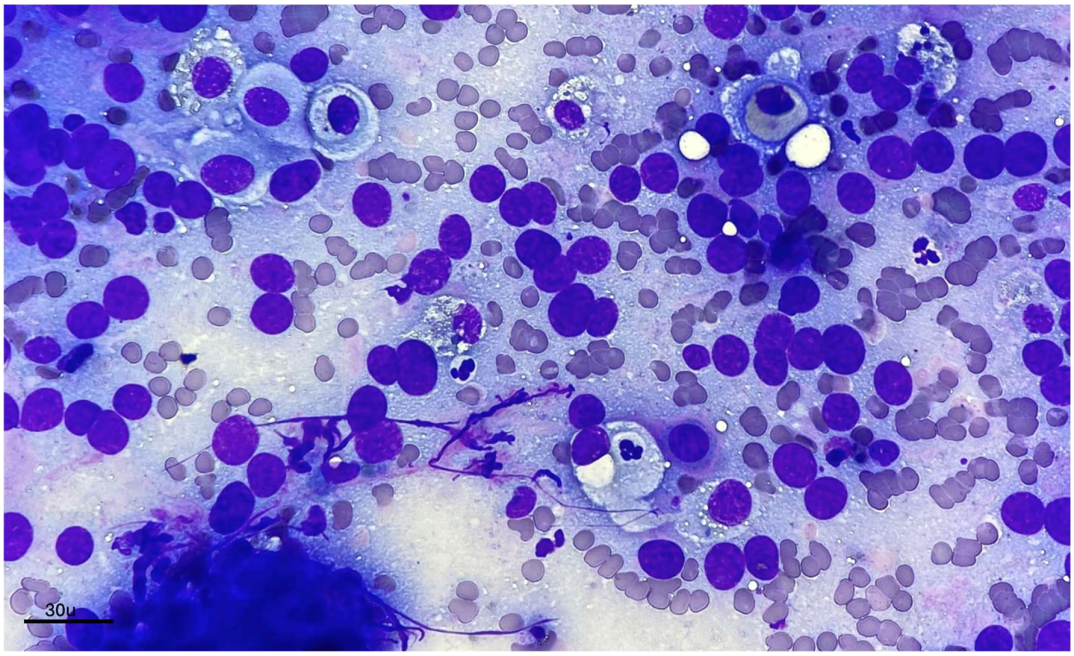

Figure 2. *Fine needle aspirate of the cervical region, (Modified Wright Giemsa) (100x)*

Questions

1. Based on the initial cytological findings, what are the main differential diagnoses for the observed cell population?
2. Considering the cytological interpretation and the location of the lesion, what would be the next recommended diagnostic steps to further investigate this case?

1. **Based on the initial cytological findings, what are the main differential diagnoses for the observed cell population?**

Cytological examination of the cervical area revealed high cellularity with moderate cell morphology preservation, a proteinaceous background with blood, cellular debris and naked nuclei. A neoplastic population of epithelial cells was observed, arranged singly or in loose cohesive clusters. These cells had poorly, sometimes well-defined borders, were round to elongated or polygonal, with light blue to pink cytoplasm containing occasional punctate vacuoles, sometimes perinuclear. Nuclei were paracentral to eccentric with finely stippled to coarse chromatin and prominent, sometimes multiple nucleoli. Anisocytosis and anisokaryosis were moderate, and nucleus:cytoplasm ratio, moderate to high. Bi- and multinucleated cells, and occasional mitoses were seen (not depicted in images). Neutrophil cannibalism/emperipolesis was rarely observed. Macrophages with vacuolated cytoplasm and phagocytized debris were present.

The cytological interpretation was a malignant neoplasia, most likely of epithelial origin. Due to the location and cytomorphology of the main population, the differentials considered included a neoplasia with neuroendocrine appearance (e.g. carotid body paraganglioma, thyroid neoplasia) or metastasis from a neoplasia located in the region that drains to retropharyngeal lymph node (e.g. oral - squamous cell carcinoma (SCC), melanoma -, salivary gland, sinonasal).

2. **Considering the cytological interpretation and the location of the lesion, what would be the next recommended diagnostic steps to further investigate this case?**

The next recommended diagnostic steps considered in this case were advanced imaging and targeted surgical biopsies to identify the primary tumor and assess the extent of disease.

After the cytological diagnosis, a CT scan was performed. The swelling of the affected cervical region corresponded to a right medial retropharyngeal lymphadenopathy, with suspicion of neoplasia (primary, metastatic, or multicentric) or lymphadenitis (Figure 3). Reactive left medial retropharyngeal and right cervical lymph nodes were also noted. No definitive abnormalities were observed in the oral cavity, teeth, hard palate, mandible, maxillary bone, nasal cavity, cribriform plate, orbits, globes, retrobulbar regions, frontal sinuses, sphenoidal recesses, calvarium, brain, temporomandibular joints, nasopharynx, tympanic bullae, or external ear canals. The thorax showed no evidence of metastasis.

Although the initial CT report did not describe any abnormalities in the oral cavity, including the tonsillar region, a retrospective reassessment of the images—performed after the case was written and prompted by the review process—revealed subtle changes in the affected tonsil (Figure 4). This reassessment was carried out in collaboration with a board-certified radiologist and identified findings consistent with the final diagnosis.

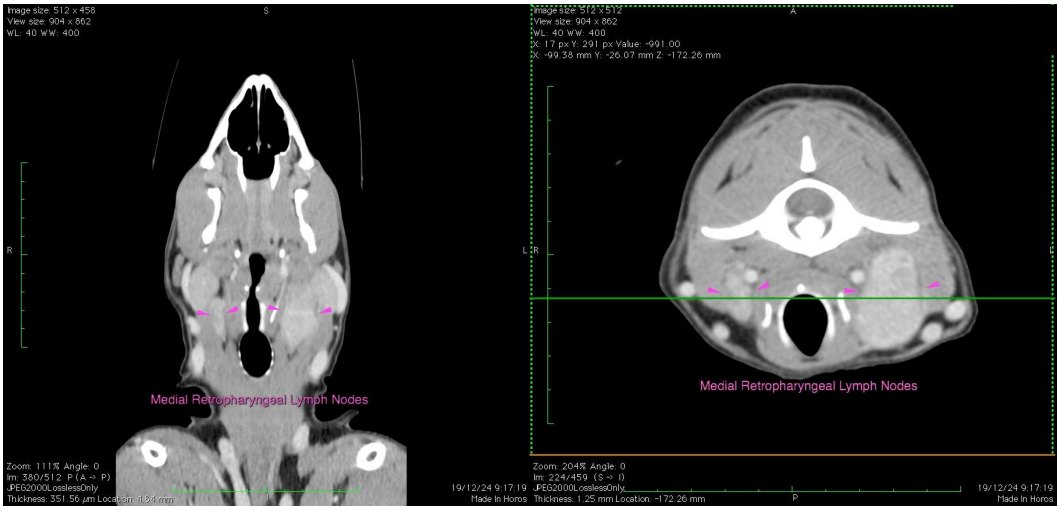

Figure 3. Head computed tomography (CT) images of the patient. Left: coronal view. Right: transverse view. The medial retropharyngeal lymph nodes are indicated in both views (pink arrowheads), with marked enlargement of the right lymph node and mild changes in the left lymph node.

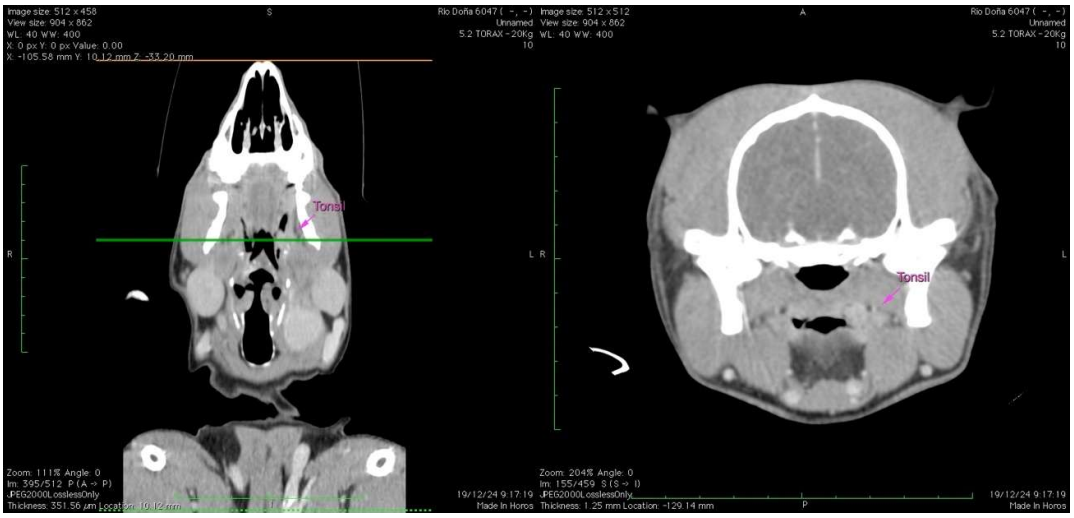

Figure 4. Head computed tomography (CT) images of the patient. Left: coronal view. Right: transverse view. The right tonsil is indicated in both views (pink arrows), which appears mildly enlarged.

Right retropharyngeal lymphadenectomy was performed. The histopathology revealed a neoplasia that exhibited a squamous differentiation pattern multifocally (Figure 5), suggesting carcinoma with squamous differentiation, being SCC as the most likely diagnosis. Given the presence of an epithelial neoplasm in the regional lymph node, metastasis from a primary neoplasm in the oral cavity (e.g. gum, tonsil, etc.) or salivary gland was considered.

Due to the histological suspicion of SCC in the retropharyngeal lymph node, a right tonsillectomy was performed to investigate the tonsil as a potential primary site, despite its normal gross and CT appearance. Although tonsillar SCC is uncommon, the tonsil is a known site of primary SCC capable of early lymphatic metastasis, and its anatomic

drainage to the medial retropharyngeal lymph node made it a plausible source. Other possible primary sites, such as the nasal cavity and salivary glands, were considered less likely based on imaging. Histopathology revealed a poorly demarcated, non-encapsulated, locally infiltrative epithelial neoplastic proliferation occupying the tonsillar mucosa and compressing the adjacent salivary gland (Figure 6). Neoplastic cells formed solid nests separated by fibrovascular stroma, with scattered foci of squamous maturation and keratin formation. Neoplastic cells were polyhedral with abundant pale cytoplasm, poorly defined borders, and oval nuclei with finely stippled chromatin and occasional nucleoli, exhibiting high atypia and evident cellular undifferentiation. Twenty-four mitotic figures were counted in 2.37 mm^2. Lymphatic invasion was observed focally in the examined sections. The neoplastic cells reached the excision margin.

Periodic acid-Schiff (PAS) staining revealed no PAS-positive mucus secretion by the neoplastic cells hence mucoepidermoid carcinoma of the salivary gland could be ruled out, although other salivary carcinomas could not be excluded.

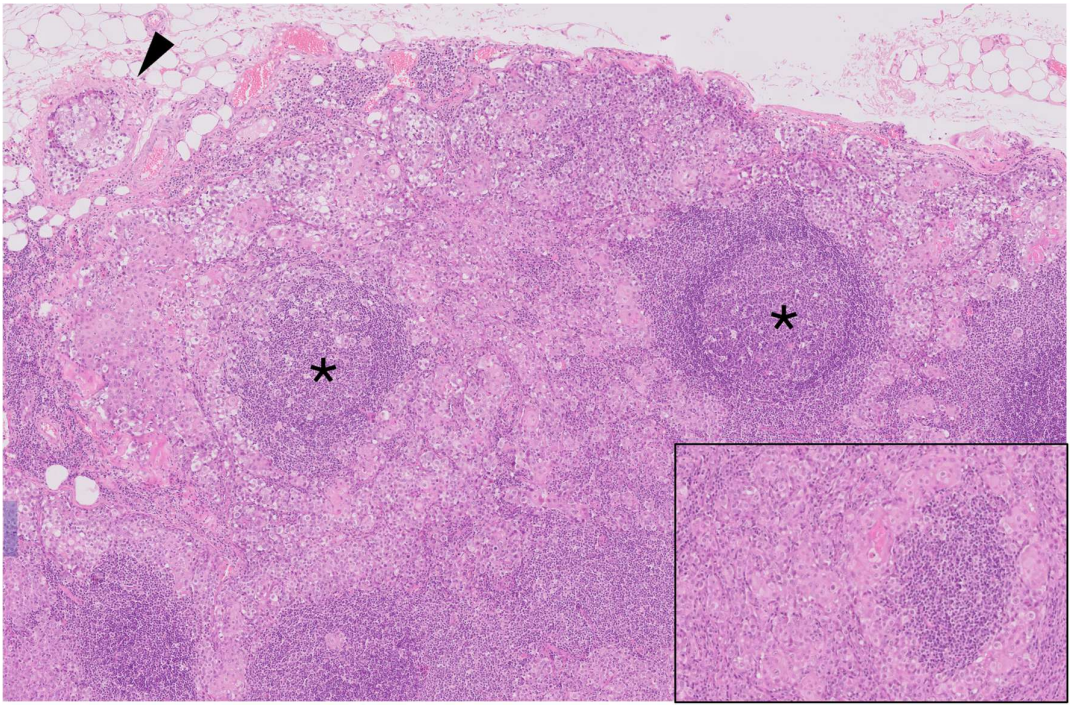

Figure 5. Right retropharyngeal lymph node (Hematoxylin-eosin). The lymphoid follicular architecture was mildly retained (asterisk). Nest and trabecula of neoplastic proliferation was infiltrating the subcapsular and medullar sinus. Subcapsular lymphoid vessel infiltration was observed (arrowhead). (10x) Inset: Highly pleomorphic neoplastic cells with squamous differentiation. (40x)

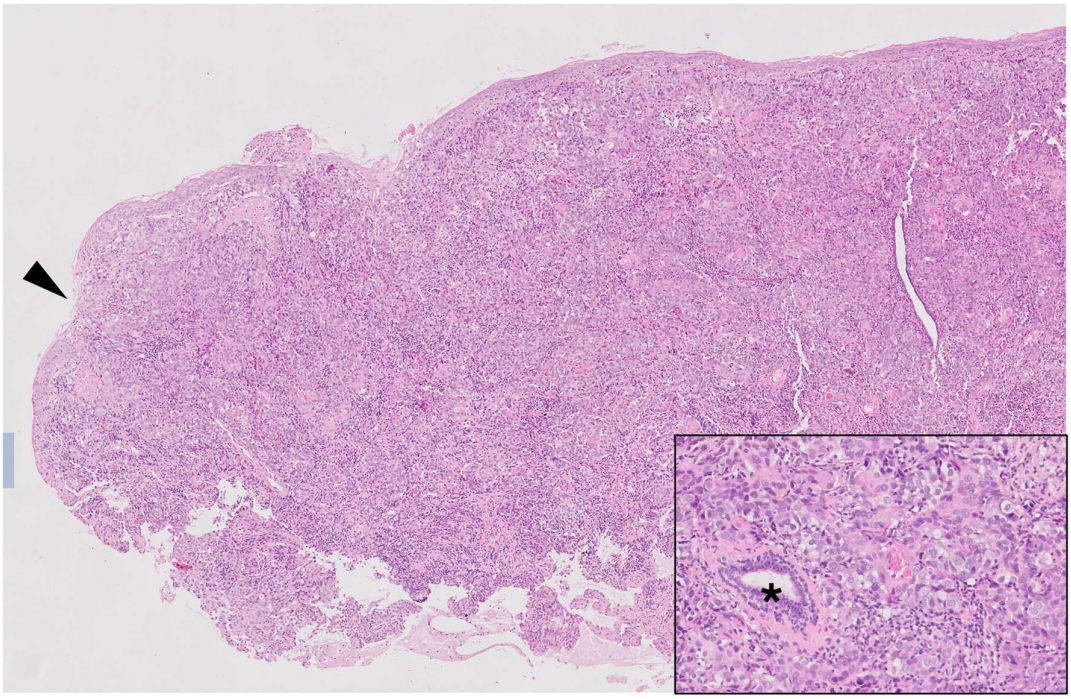

Figure 6. Tonsil (Hematoxylin-eosin). Squamous cell carcinoma with superficial mucosa invasion (arrowhead) (10x). Inset: Highly pleomorphic neoplastic cells with squamous differentiation. Remnant salivary gland duct (asterisk) (40x)

Interpretation/Diagnosis

The definitive diagnosis was infiltrative tonsillar squamous cell carcinoma. However, salivary gland squamous cell carcinoma remained as the main alternative differential diagnosis, as it could not be completely ruled out due to the proximity to the accessory salivary gland. The poor differentiation suggested a more aggressive behavior.

Additional information

To better define the anaplastic groups in the lymph node and to contrast the cytological appearance of the neoplasia, immunohistochemistry (IHC) for cytokeratin (CK) and synaptophysin was requested. Results showed negative synaptophysin staining in the neoplastic cells, confidently ruling out a neuroendocrine origin. CK was positive in 70% of the neoplastic population (intense cytoplasmic positivity) and weak/sparse or negative in the remaining 30%, confirming the epithelial origin (Figure 7). Vimentin staining was negative in all neoplastic cells, with positivity observed only in the surrounding stroma/fibrosis (Figure 8).

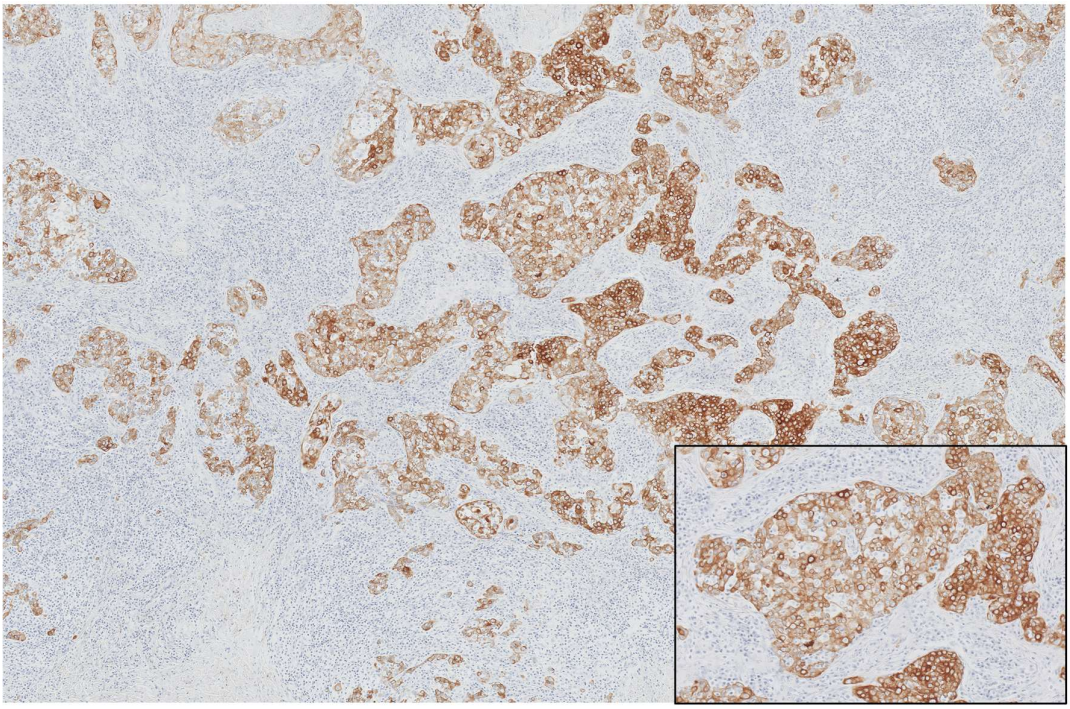

Figure 7. Right retropharyngeal lymph node (Pan-cytokeratin IHC). Neoplastic cells showed moderate to intense cytoplasmic immunoreaction. (20x) Inset (40x)

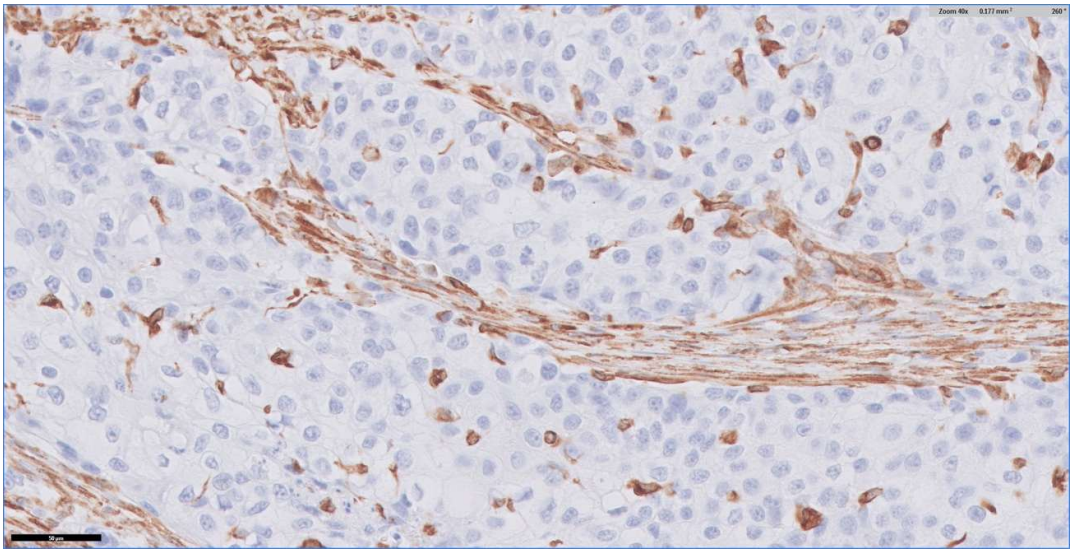

Figure 8. Right retropharyngeal lymph node (Vimentin IHC). Vimentin staining was negative in neoplastic cells, with positivity observed only in the surrounding stroma/fibrosis. (100x)

Follow up and clinical outcome

Electrochemotherapy was administered following tonsillectomy, combined with a chemotherapy protocol consisting of daily NSAIDs (firocoxib at 5 mg/kg) and toceranib (2.2 mg/kg, Palladia) given 3 times a week.

Three months after the initial visit, during re-staging, splenic cytology revealed a malignant epithelial neoplasm with features similar to those seen in the lymph node (Figure 9), correlating with multifocal hypoechoic lesions on ultrasound. This finding suggests distant metastasis, consistent with the aggressive behavior of tonsillar squamous cell carcinomas.

Due to clinical worsening of the patient and the lack of efficacy of the treatment, carboplatin was recommended, but the tutors declined it and decided to perform humanitarian euthanasia. The time elapsed since the patient's first visit was approximately 4 months.

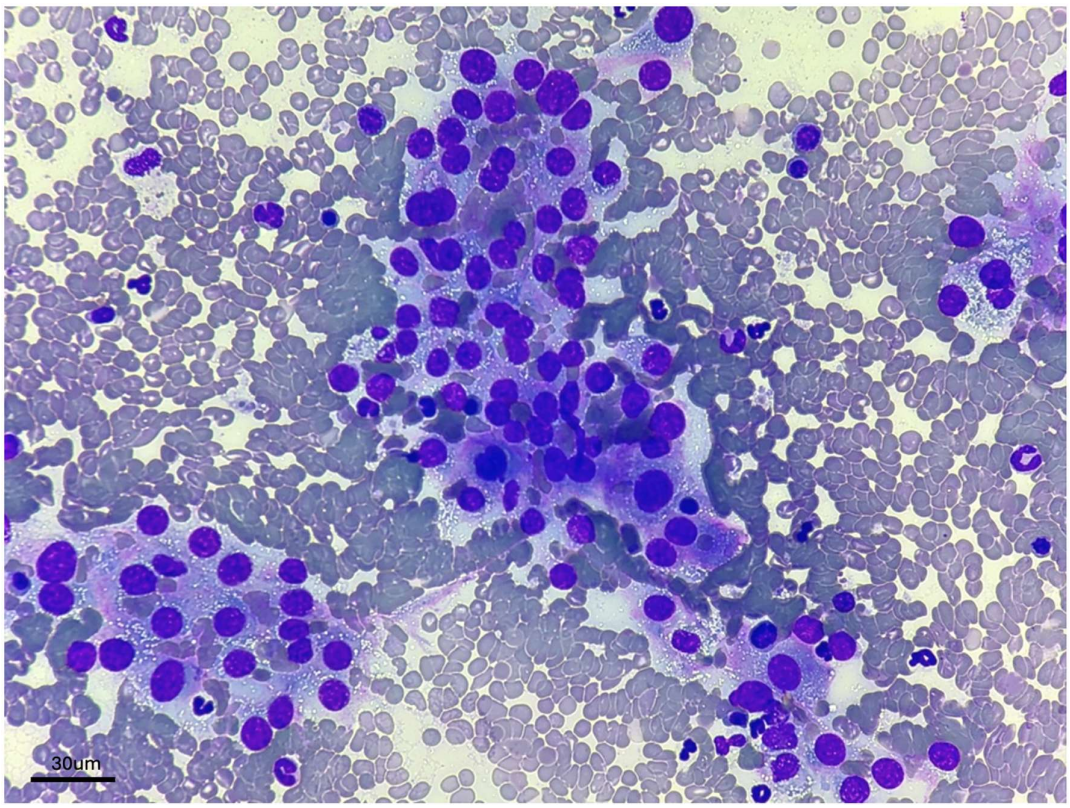

Figure 9. *Fine needle aspirate of the spleen, (Wright Giemsa) (40x).*

Discussion

Canine tonsillar squamous cell carcinoma (TSCC) is recognized as a relatively uncommon yet aggressive oral neoplasm with a high potential for metastasis.[1] TSCC is less commonly reported in dogs compared to non-tonsillar squamous cell carcinomas (SCC), but it shows a significantly higher tendency for local metastasis (up to 73%)[2]. The disease typically spreads to regional lymph nodes (mandibular, retropharyngeal, cervical) and the lungs and, rarely, to spleen.[2]

The sequence of diagnosis in this case —first identifying nodal metastasis and later confirming the tonsillar origin— highlights the occasionally subtle or asymptomatic presentation of TSCC and its high metastatic potential. Despite surgical excision (tonsillectomy and lymphadenectomy), electrochemotherapy and adjuvant therapy with a tyrosine kinase inhibitor and a non-steroidal anti-inflammatory drug, the dog's clinical course was rapidly progressive, with euthanasia performed approximately 120 days after diagnosis. This outcome is consistent with previous findings which stated that initial staging significantly influences prognosis.[3] In particular, dogs presenting with metastasis at diagnosis had median survival times (MSTs) of 134 days for locoregional metastasis and 75 days for distant spread, compared to an MST of 637.5 days in dogs with unilateral tonsillar involvement and no evidence of metastasis at initial staging.[3] In this case, the presence of nodal metastasis at diagnosis likely contributed to the poor outcome, despite the use of multimodal therapy.

The diagnosis of tonsillar neoplasia can be challenging. Imaging studies of tonsillar tumors have shown that, while affected tonsils are typically enlarged, there are instances where they may appear normal in size or only slightly enlarged.[4] This discreet presentation can sometimes lead to missed diagnoses, as the changes may not be easily detectable. In the case described, the initial physical examination and CT scan did not reveal any abnormalities in the tonsils, and cytological findings from the cervical region did not display the typical cytomorphological features of SCC[5] that would have indicated a probable oral or tonsillar origin for the tumor. It was only after the histological examination of the affected lymph node that tonsillectomy was pursued due to the probable origin of the neoplasia. However, it is suggested that tonsillar neoplasia should be considered in the differential diagnosis of isolated medial retropharyngeal lymphadenomegaly, even with a normal-sized tonsil.[4]

The initial cytological findings suggested a neuroendocrine origin due to the appearance of the neoplastic cells, highlighting the diagnostic challenge. However, the histopathological examination revealed that, although the pattern of nests and cellular morphology could be indicative of a neuroendocrine neoplasm, squamous differentiation is not a feature typically associated with neuroendocrine tumors, making this diagnosis unlikely. The cervical mass was definitively diagnosed as nodal poorly differentiated metastatic SCC. The suspicion is that the cytological findings corresponded to these less differentiated areas of the neoplasm. Similarly, in the spleen cytology performed later, the diagnosis of SCC solely based on cytology would have been difficult without considering the prior history.

The main alternative differential diagnosis in this case was a neoplasm originating from the salivary gland, being a metastasis from locations that drain into the retropharyngeal lymph node (e.g., sinonasal region—such as squamous cell carcinoma or adenosquamous carcinoma) less probable due to the absence of lesions in those areas. A salivary gland neoplasm was the leading suspicion, as histopathologic examination of the tonsil revealed proximity to the accessory salivary gland. Among salivary gland tumors, mucoepidermoid carcinomas are characterized by proliferations of squamous cells, vacuolated mucus-secreting cells, and intermediate cells.[1,6] The squamous cells rarely produce keratin, but intercellular bridges can be identified. The mucus-secreting cells and intracellular mucus can be confirmed using mucicarmine, alcian blue, or PAS stains. In this case, the PAS stain was negative, ruling out this type of neoplasm. Other salivary gland tumors to consider include squamous cell carcinomas, although these are rare, and care must be taken to exclude invasion of the salivary gland by an SCC originating elsewhere in the oral cavity.[1]

Therefore, despite already having the diagnosis, IHC was requested for scientific purposes to exclude a potential neuroendocrine origin. The positive immunostaining for cytokeratin in the majority of the neoplastic cells and the negative immunostaining for synaptophysin supported the epithelial origin and made a neuroendocrine carcinoma less likely. In humans, conversion from SCC to neuroendocrine carcinoma of the esophagus has been described following definitive chemoradiotherapy, with confirmation using synaptophysin and other markers. While this phenomenon was considered highly unlikely in this case, IHC helped to rule it out.[7]

Interestingly, the presence of cytokeratin-negative tumor cell nests in a carcinoma with confirmed metastasis (lymph nodes and spleen) suggests that these cell populations may represent a more aggressive, dedifferentiated subclone with enhanced metastatic ability. This aligns with findings in human oncology, where loss of cytokeratin is linked to advanced stage, metastatic spread, and poor prognosis in some eptihelial neoplasms.[8-11]

Also, in both canine and feline epithelial tumors, loss or reduction of cytokeratin immunoreactivity is frequently associated with epithelial-mesenchymal transition (EMT). EMT is a process by which epithelial tumor cells lose their epithelial markers (such as cytokeratins and E-cadherin) and gain mesenchymal traits (such as vimentin), facilitating invasion and metastasis.[12] While this case specifically notes cytokeratin loss in a subset of cells, vimentin immunostaining was negative in the neoplastic population, providing no evidence of mesenchymal transition in these areas.

There are some limitations in this case, acknowledging certain diagnostic and clinical challenges encountered during the diagnostic workup and treatment. Cytological examination of the tonsil prior to lymphadenectomy would have saved surgical time for the animal. Despite the attempt to better define the anaplastic foci through IHC, it was not possible to consistently rule out the presence of another concurrent neoplasm, although it was considered unlikely. Given the presence of splenic metastasis, pulmonary metastasis was also probable, despite not being detected on thoracic imaging. Also, the tonsillar alteration was not identified on the initial CT interpretation. This may be partly

attributed to the inherently subtle imaging features of some tonsillar tumors. Additionally, human factors such as workload, time constraints, or diagnostic fatigue may have contributed to the oversight. While retrospective review revealed changes in the affected tonsil consistent with the final diagnosis, this case underscores the importance of maintaining a high index of suspicion in patients with compatible clinical signs, even in the absence of clear imaging findings. It also highlights the value of collaborative image review and the potential role of follow-up evaluations in complex or ambiguous cases.

In conclusion, this case illustrates a typical and aggressive presentation of canine TSCC exhibiting atypical cytomorphology. It highlights the potential diagnostic shortcomings of imaging in identifying the primary tumor, especially when changes are subtle. The case also emphasizes the importance of histopathology in differentiating SCC from other neoplasms. The rapid progression underscores the often-guarded prognosis associated with this type of cancer in dogs.

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