GASTRIC INTRAVASCULAR LYMPHOMA IN A DOG: CASE REPORT AND LITERATURE REVIEW

Abstract

Intravascular lymphoma (IVL) is a rare, high grade, extranodal lymphoma characterised by selective proliferation of neoplastic lymphocytes within the lumen of small vessels. A 10-year-old, female intact mixed breed dog was presented with a 7-month history of vomiting and anorexia. Physical examination revealed abdominal discomfort. Ultrasonography and endoscopy identified a submucosal gastric mass. Excision was performed by partial gastrectomy and histopathology and immunohistochemistry confirmed a T-cell IVL. The owner declined chemotherapy, and the dog was instead treated palliatively with prednisolone. Two months post-surgery, vomiting recurred and abdominal ultrasonography revealed a large gastric ulcer with focal peritonitis. The dog was euthanised four months after initial presentation and *post mortem* examination confirmed IVL recurrence in the stomach and an isolated nodule of neoplastic cells in the omentum. No involvement of other organs was found following histopathological examination. This is the first description of primary gastric intravascular lymphoma causing chronic vomiting in a dog.

Introduction

Gastric neoplasia accounts for less than 1 % of cancer in dogs, and carcinoma is the most common malignancy in this location.[1,2] Other gastric tumours include leiomyoma, leiomyosarcoma, lymphoma, extramedullary plasmacytoma, mast cell tumour and histiocytic sarcoma.[2] Gastrointestinal lymphoma is the most common form of extranodal lymphoma in dogs and involves the stomach in 16-40% of the cases.[3,4] T-cell lymphomas are more prevalent and the disease is normally associated with a poor prognosis, with survival times ranging from 13 days to 14 weeks, due to a limited response to chemotherapy.[4] Intravascular lymphoma (IVL) is included in the canine WHO classification of lymphoid neoplasia as an uncommon, high grade, extranodal, subtype of lymphoma characterised by intravascular localisation of the neoplastic lymphocytes on histopathology.[3,10] IVL has been reported in only 25 dogs, 2 cats and 1 horse.[5-12,18] In the majority of cases, pronounced tropism for the central nervous system (CNS) results in progressive neurological deficits which are often multifocal.[9-11] Given that there are difficulties in biopsying CNS lesions, the diagnosis is often made *post mortem*. In all cases reported in dogs, IVL has been associated with an aggressive behaviour and a poor prognosis.[5-12] To our knowledge, this is the first report of primary gastric IVL in a dog.

Case history

A 10-year-old, female intact mixed breed was presented with a 7-month history of vomiting, anorexia and intermittent signs of abdominal pain. Vomiting occurred daily, mainly after food ingestion. Previous treatment included ranitidine (150mg *per os* q12h) and metoclopramide (10mg *per os* q8h) with only transient improvement of the clinical signs, and no response to feeding a low-fat highly digestible diet for 1 month. On physical examination, the dog was underweight (body condition score [BCS]: 3/9, 10.2Kg) and discomfort was present during abdominal palpation, but no other abnormalities were detected. Haematology revealed moderate thrombocytosis (566 $x10^9$/L reference interval [RI]: 150-400). Abnormalities in serum biochemistry included moderate hypoalbuminaemia (19.7g/L RI: 26.3-38.2), mild hyperglobulinaemia (44.6g/L RI: 23.4-42.2) with normal total protein concentration (64.3g/L RI: 54.9-75.3), mild hyperkalaemia (6.08mmol/L RI: 3.6-5.6), total hypocalcaemia (2.24mmol/L RI: 2.36-2.84) and hyperphosphataemia (1.71mmol/L RI: 0.8-1.6).

Thoracic radiographs were unremarkable, whereas abdominal radiographs revealed gastric wall thickening and mild small intestinal distention. Ultrasonography confirmed a hypoechoic, thickened and irregular gastric wall with loss of layering in the fundic region (*Figure 1*). Regional lymphadenopathy was noted, affecting the splenic (17x12mm), gastric (7x7mm) and hepatic (19x11mm) lymph nodes. A hyperechoic nodule in the spleen (6x4mm) and another nodule in the liver (8x6mm) were also identified. Cytological examination of fine-needle aspirates from general spleen, splenic lymph node, and gastric lymph node were all consistent with reactive lymphoid hyperplasia. Gastroscopy confirmed

a large, rounded, irregular, submucosal mass in the lumen of the gastric fundus, between the *angularis incisura* and *cardia* (*Figure 2*). Multiple grab-biopsy samples were taken from both normal and abnormal gastric mucosa. Pending results, the patient was discharged with maropitant (24mg *per os* q24h), omeprazole (10mg *per os* q24h), paracetamol (200mg *per o*s q8h) and mirtazapine (15mg *per os* q24h).

Histopathological examination of the gastric mass revealed a monomorphic proliferation of spindle cells which exhibited mild-to-moderate anisokaryosis. Immunohistochemistry for pan-cytokeratin (pan-CK) and c-kit was negative, but neoplastic cells stained positively for alpha smooth muscle actin (α-SMA), suggesting a mesenchymal neoplasm, most likely a leiomyosarcoma. Based on the staging undertaken, the lesion was thought potentially resectable and the dog underwent tumour excision via partial gastrectomy with approximately 2cm lateral margins excision. The final histopathology report confirmed that below a large and deep chronic ulcer of the mucosa, there was a proliferation of atypical, large round cells (20 to 30µm in diameter) consistently located within the lumen of vessels throughout the submucosa and extending to the *muscularis* (*Figure 3 a and b*). These cells exhibited moderate degree of cellular atypia, and mitotic index of 20 x 10 high power fields. Numerous acute and chronic thrombi, neoangiogenesis, oedema and vascular necrosis were associated with vascular occlusion. In co-localisation with the ulcer, vessels engorged with neoplastic round cells were also observed along the serosa and focally extending to the omentum. The neoplastic round cells were positive for CD3 and negative for pan-cytokeratin, Pax-5 and CD79α, confirming the diagnosis of T-cell intravascular lymphoma (IVL) (*Figure 3 c and d*).

The patient´s clinical signs completely resolved after surgery. Adjunctive chemotherapy options were declined by the owner due to financial constraints and the dog was continued on palliative treatment with prednisolone (10mg *per os* q24h) and omeprazole (10mg *per os* q24h).

Two months after surgery, the dog returned to the hospital due to recurrence of vomiting. Ultrasonography revealed a large defect within the fundic stomach wall (19 x 25mm in diameter and 15mm depth) consistent with a gastric ulcer. There was marked thickening of the wall (up to 16mm) and loss of layering in both sides of the ulcer, indicating tumour recurrence. The adjacent mesentery was hyperechoic and contained a small pocket of free fluid, suggesting focal peritonitis. The nodular lesions in the liver and spleen and lymphadenopathy were similar to the previous study. Due to the poor prognosis associated with this subtype of lymphoma, the early recurrence and the risk of imminent stomach perforation the dog was euthanised four months after diagnosis.

Post mortem examination of the stomach confirmed the presence of recurrent masses within the gastric wall associated with large mucosal ulcerations (*Figure 3 e and f*). Microscopically, the presence of neoplastic cells within the vascular lumina was associated with occlusive thrombi and consequent ischemic lesions and endothelial damage. A single extra-gastric focus of intravascular lymphoma was detected in the omentum, close the serosa of the stomach. Given that the post-surgical histopathology examination reported extension of the neoplastic cells to the omentum, this omental nodule likely reflected residual disease due to incomplete excision. Histopathological examination of the thyroid,

adrenal glands, brain, heart, lungs, liver, spleen, bone marrow, tonsils, pancreatic and gastric lymph nodes, pancreas, small intestine, colon, kidneys, urinary bladder, ovaries and peripheral nervous tissue (including both sciatic nerves and brachial plexuses) showed no evidence of IVL.

Discussion and literature review

Intravascular lymphoma is a rare, high grade, extranodal lymphoma characterised by monoclonal proliferation of lymphocytes within the lumen of vessels.[3,10] The diagnosis of IVL is challenging due to the non-specific clinical signs, the absence of peripheral lymphadenopathy and the fact that neoplastic cells are rarely found in the peripheral blood.[6] The mechanism by which these tumours arise and why neoplastic lymphocytes remain confined to the local vascular lumina remains uncertain.[13,18] Some authors suggest that the presence of vascular microthrombi could cause occlusion of the vessels and limit the dissemination of neoplastic cells within the vasculature,[13] but this theory is unlikely given that most dogs die with disseminated disease. More recently, abnormalities in the molecules involved in lymphocyte and endothelial adhesion has been speculated due to the lack of β1 and β2 integrins in some human IVL, both which are essential for transvascular migration.[10] A recent study showed that canine IVL strongly expressed CD44 and, more inconsistently CD29, suggesting cell surface adhesion receptors could play a role in the formation of lymphocyte aggregates.[18]

In contrast to humans, where IVL is classified as a non-Hodgkin's, diffuse large B-cell lymphoma in 90% of cases, in dogs it seems to have a predominant T-cell immunophenotype, with null cell phenotypes being more common that B-cell phenotypes.[10] However, this has been recently questioned in a small case series where both B and T cell immunophenotype were found to be equally prevalent.[18] Immunopositivity for CD3 in this case indicated T cell origin.

Neurological signs, including ataxia, paresis and vestibular deficits, are reported in up to 88% of dogs with IVL.[10] Despite the tropism of this tumour for nervous tissue, no neurological signs were reported in this patient and histopathological examination of the brain, spinal cord and peripheral nerves at post mortem revealed no neoplastic cells. In humans, other common clinical signs include skin lesions (erythema and eruptions), fatigue or pyrexia of unknown origin, but these seem to be rare in animals.[15] Vomiting, haematochezia and melena are uncommonly reported in humans.[16-17]

Clinico-pathological findings in cases of IVL are normally non-specific but may reflect organ involvement. The thrombocytosis seen in this case could reflect intermittent gastrointestinal bleeding from the tumour or stress-related increase in endogenous steroids due to chronic vomiting. Hypoalbuminemia occurs as a consequence of neoplastic infiltration causing gastric barrier disruption. This abnormality was found in 80% of dogs with gastrointestinal lymphoma and is a negative prognostic factor for these patients.[2,3] The ultrasonographic findings in dogs with gastrointestinal lymphoma can vary widely and include irregularities in the mucosal surface, changes in wall thickness and layering, variable echogenicity and presence of regional lymphadenopathy, but no pathognomonic sign exists.[20] Interestingly, one study identified lymphoma as the most common gastric neoplasia missed on ultrasonography.[20]

Given these limitations, endoscopy is the preferred non-invasive method to obtain a diagnosis. In this case, the endoscopic appearance of the tumour differs from the ones reported in humans, where polypoid lesions predominate.[16,17] The misdiagnosis of

leiomyosarcoma on the endoscopical biopsies likely reflects non-representative sampling of highly-reactive spindloid myofibroblasts and fibroblasts attempting to heal the superificial portion of the ulcerated mucosa, while the primary population of neoplastic cells was "hidden" deep within the submucosa and muscularis. The limitations associated with endocopically-guided biopsies are well known:[4] in one study, endoscopically-guided biopsies in dogs and cats with gastrointestinal lymphoma produced accurate results in only 59% of the cases.[20]

In the majority of dogs reported to have IVL, diagnosis was achieved at *post mortem* examination. Only two cases have been described where an *ante mortem* diagnosis was made in dogs that underwent skin and brain biopsies, respectively.[9,14] Unlike the current case, all previously reported dogs with IVL undergoing full *post mortem* examinations have been found to have widespread dissemination of the tumour.[5-12]

The difficulty in obtaining a prompt diagnosis, the rarity of the disease and the rapid progression of IVL can make appropriate invervention very difficult.[15] The only two dogs where diagnosis was achieved *ante mortem* deteriorated within 2 and 4 weeks due to cancer progression.[9,14] The dog in this case report survived 4 months, which likely reflects the localised presentation and the lack of central nervous system involvement in our patient. Surgical debulking of the local disease likely extended survival. Unfortunately, there is little information available about chemotherapy for IVL in dogs.[9]

In the current case, the owners declined post-operative chemotherapy. There is only one case report of a dog with intracranial IVL treated with chemotherapy (L-asparaginase and vincristine), and no clinical response was observed.[9] In humans, chemotherapy with CHOP (vincristine, cyclophosphamide and doxorubicin) is reasonably effective with complete responses in 55% of the cases, with some patients achieving prolonged periods of disease-free survivals.[15] The addition of rituximab, high-dose methotrexate and cytarabine in cases with central nervous involvement provides clinical benefit, with disease free intervals ranging from 14 months to 2 years.[19] The role of radiotherapy in the management of IVL is still poorly defined but could help with tumour control in localised cases.[19]

The formation of microthrombi within the tumour, as it was seen in the histopathology of our dog, may contribute to tissue hypoxia and play a role in chemoresistance by difficulting chemotherapy drug penetration within the tumour.[23] The presence of fibrin thombi is commonly described in the histopathology findings from humans with IVL and recent studies have suggested a potential benefit in treating these patients concurrently with unfractionated heparin based on the potential expression of heparin-responsive adhesion molecules, such as L-selectin, in neoplastic lymphocytes responsible for IVL.[21-23] Additionally, unfractionated heparin has been shown to inhibit p-glycoprotein-mediated multidrug resistance *in vitro* and improves survival in cancer patients with a pro-thrombotic state. Thus, treatment with heparin could prevent the aggregation of the neoplastic lymphocytes within vascular lumen, thereby reducing the formation of microthrombi within the tumour and facilitating chemotherapy drug penetration.[19,23]

Despite the available treatments, human IVL is associated with a mortality rate of 80%.[15] This reflects the need for development of a diagnostic algorithm and serum biomarkers to help provide a prompt diagnosis and rapid treatment initiation in these cases.

Conclusion

Intravascular lymphoma should be considered as a rare differential for gastric tumours. The localised presentation and the lack of central nervous system involvement could be associated with longer survivals as demonstrated in the current case. IVL should still be approached as a systemic disease and a poor prognosis should be expected, particularly in the absence of systemic therapy.

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FIGURE. 1. Ultrasonographic image of the stomach of a 10-year-old, female intact mixed breed dog with gastric intravascular lymphoma: the gastric wall in the fundic region is focally thickened (21.6mm in maximum diameter) and hypoechoic with loss of layering (arrow) compared to the rest of the stomach (arrowhead).

FIGURE. 2. Gastroscopy images of the stomach of a 10-year-old, female entire mixed breed dog with intravascular lymphoma. A large, ulcerated submucosal mass was found in the pyloric region of the stomach.

FIGURE. 3. Gross and histopathological findings of intravascular lymphoma in the stomach of a 10-year-old, female entire mixed breed dog. **A.** Submucosa and muscularis are almost completely composed of numerous vessels engorged by large atypical round cells and occluding thrombi (100X, scale bar 200µm). **B.** Monomorphic population of round atypical cells, among which some are in mitosis, occupy the vascular lumina (400X, scale bar 25µm). **C.** The majority of intravascular atypical round cells are CD3 positive (200X, scale bar 50µm). **D.** CD79a positive cells are scattered within the interstitium (200X, scale bar 50µm). **E.** Post-mortem image of the stomach showing one of two large foci of mucosal thickening with central large depressed ulcer. **F.** Post-mortem examination of the stomach. On cut surface, wall thickness is increased and layers obscured by the ulcerated mass.