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Gastrointestinal B-lymphoblastic lymphoma in a dog: a case report

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ABSTRACT: A four-year-old Bullmastiff weighing 44 kg was presented with a 14-day history of weight loss, vomiting and diarrhoea. Abdominal ultrasonography showed the presence of abdominal lymphadenopathy and thickening of the wall of the descending colon. Esophagogastroduodenoscopy and colonoscopy with biopsy were performed. Histological examination revealed a high-grade lymphoblastic lymphoma, flow cytometric analysis detected malignant cells of the immature B phenotype. PCR for antigen receptor rearrangement confirmed IgH monoclonality pointing together with immunophenotyping to B-cell lymphoma. The dog was treated using a multi-agent chemotherapy protocol. The overall survival time was 487 days. This was an unusual case of primary gastrointestinal B-lymphoblastic lymphoma in a dog with survival equivalent to that of the multicentric form.

Keywords: canine; alimentary; lymphoid neoplasia; immunophenotyping; PARR; chemotherapy

List of abbreviations:

B-LBL = B-lymphoblastic lymphoma, **FC** = flow cytometry, **FNAB** = fine-needle aspiration biopsy, **GI** = gastrointestinal, **LPE** = lymphocytic-plasmacytic enteritis, **PARR** = polymerase chain reaction for antigen receptor rearrangement

Lymphoma is the most common haematopoietic malignancy in dogs (Vail and Young 2007).

The alimentary form accounts for approximately 10% of all canine lymphomas (Mortier et al. 2012). The small intestine is most frequently involved, followed by the stomach (Coyle and Steinberg 2004).

Most cases of primary gastrointestinal (GI) lymphomas are of T-cell origin (Marconato et al. 2011).

The response to multi-agent chemotherapy protocols is poor and survival time is short in most cases (Frank et al. 2007).

Case description

In April 2006, a four-year-old male Bullmastiff weighing 44 kg was presented to the Clinic of Dog and Cat Diseases at the University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic. The primary clinical complaint was a history of weight loss (12%), lethargy, vomiting and diarrhoea lasting for 14 days. Symptomatic therapy (antiemetic agent, H_2-blocker) initiated at another veterinary clinic had failed and the patient was referred for further diagnostics. Additionally, the owner men-

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tioned that the dog had never had normal stool in his life.

Abnormalities noted on initial physical examination included poor body condition, pale mucous membranes and a palpable mass in the mesogastrium. Rectal examination revealed multiple nodular lesions.

The initial laboratory tests revealed moderate nonregenerative microcytic hypochromic anaemia (haematocrit 0.27 l/l; reference range 0.4–0.55 l/l), mild hypoproteinaemia (54.5 g/l; reference range 55–75 g/l), mild hypoalbuminaemia (25 g/l; reference range 28–40 g/l) and a mild increase in amylase activity (2068 U/l; reference range 372–1506 U/l).

Plain abdominal radiographs revealed the presence of abdominal masses at the level of the fourth lumbar vertebra (2.5 × 1 cm) and the twelfth/thirteenth thoracic vertebrae (6 × 6 cm). An obstructive pattern was not seen. Thoracic radiographs were unremarkable.

Abdominal ultrasonography showed moderate enlargement of the mesenteric lymph nodes and marked focal thickening of the wall of the descending colon, over a distance of 4 cm.

Ultrasound-guided fine-needle aspiration biopsy (FNAB) of abdominal lymph nodes for cytology was performed. Cytological examination showed a predominance of medium-sized lymphocytes with a thin rim of moderately basophilic cytoplasm. The nuclei were round or slightly irregular in shape, with finely stippled chromatin and multiple nucleoli. The mitotic index was high, with four mitotic figures per five fields under a × 40 objective.

Parasitological examination of three stool specimens on non-consecutive days was negative.

The dog was prepared for subsequent upper and lower GI endoscopy. Food was withheld from the patient for 48 hours before the procedure. A high-volume colonic lavage solution (Fortrans, macrogolum, Ipsen Pharma) and multiple enemas were administered. The patient received intravenous fluids (Ringer's solution, 3.5 ml/kg body weight/h, *i.v.*), amoxicillin-clavulanate 25 mg/kg body weight, *i.v. q* 8 h (Augmentin, GSK), famotidine 1 mg/kg body weight, *i.v. q* 24 h (Quamatel, Gedeon Richter Marketing Czech Republic) and metoclopramide 0.5 mg/kg body weight, *s.c. q* 8 h (Degan, Sandoz s.r.o.).

Endoscopic examination was performed using a flexible Olympus CF 40L endoscope. Upper GI endoscopy revealed multiple nodular lesions in the region of the cardia, angulus and pylorus, infiltrative lesions and thickening of rugae in the region of the angulus (Figure 1A) and flat infiltrative lesions in the duodenum. The oesophagus was normal. Retrograde ileoscopy revealed pronounced granularity and friability of the ileum. Colonoscopy showed multiple, variable-sized nodular lesions in the descending colon and rectum (Figure 1B). Multiple deep biopsy specimens from all accessible GI segments including the oesophagus, stomach, duodenum, ileum and colon were taken for cytology (Figure 2) and histopathology. The rapid urease test for detection of *Helicobacter* spp. infection was negative.

The patient was discharged from the hospital with the following medication: amoxicillin-clavulanate 25 mg/kg body weight, *p.o. q* 12 h, metronidazole 15 mg/kg body weight, *p.o. q* 12 h (Entizol, Zaklady Farmaceutyczne Polpharma S.A.), famotidine 1 mg/kg body weight, *p.o. q* 24 h (Famosan, Pro. Med. CS Praha a.s.) and metoclopramide 0.5 mg/kg body weight, *p.o. q* 8 h.

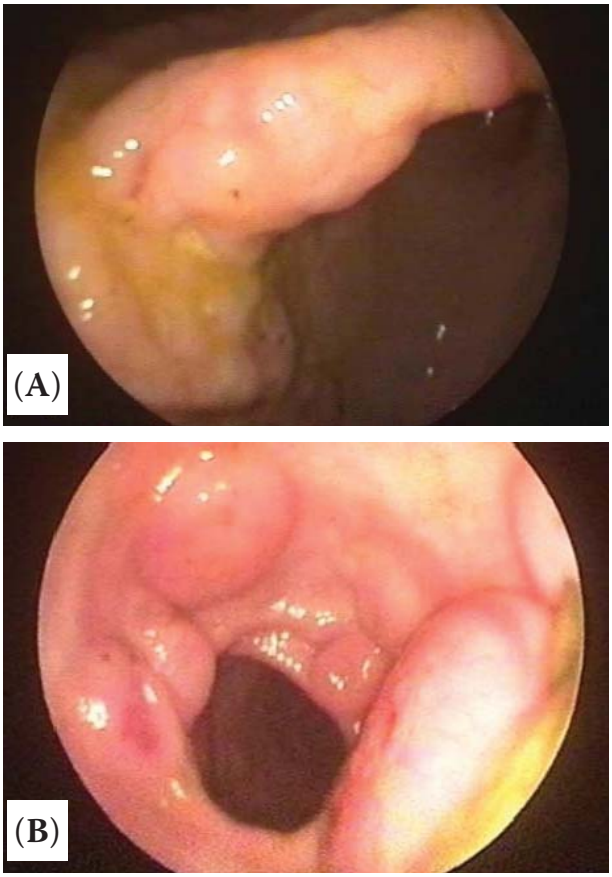

Figure 1. Endoscopic findings. (**A**) Nodular lesions in the gastric angulus. (**B**) Multiple variable-sized nodular lesions in the descending colon

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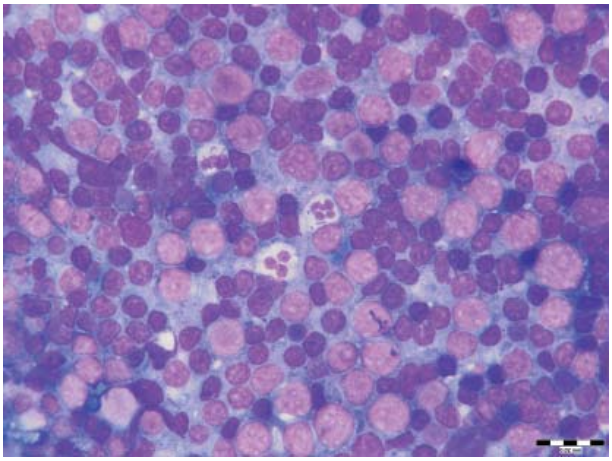

Figure 2. The imprint cytology of a duodenal biopsy specimen; predominance of small-to-medium-sized lymphocytes. The medium-sized lymphocytes have smooth-to-finely stippled chromatin and frequent variably distinct, multiple nucleolar rings. They contain scant amounts of moderately basophilic cytoplasm, which occasionally contains a small perinuclear clearing. There are low-to-moderate numbers of mixed bacteria (cocci and bacilli) in the background and within the cytoplasm of the neutrophils (Dip Quick Stain; magnification × 1000)

Endoscopy biopsy samples were fixed in 10% buffered formalin, dehydrated, embedded in paraffin wax, sectioned on a microtome at a thickness of 4 µm, and stained with haematoxylin and eosin.

Histopathological findings confirmed non-Hodgkin's high-grade lymphoblastic lymphoma (according to the World Health Organization classification criteria). A population of small to medium-sized lymphoblasts was detected, with nuclei 1.5–2 times the diameter of red blood cells, often irregular in shape, or round-to-oval, with finely dispersed pattern, indistinct or small nucleoli and a small rim of eosinophilic cytoplasm. Mitotic index was high. Lymphocytic-plasmacytic inflammation was also detected and even predominated in some sections. Neoplastic infiltration was detected in the lamina propria and in some parts of the lamina muscularis mucosae of the stomach, duodenum, ileum and colon; the submucosa of the colon was also affected (Figures 3A–3C).

Immunohistochemistry was performed using monoclonal mouse anti-human CD79α (HM57, Agilent, Santa Clara, USA, dilution 1 : 25) and polyclonal rabbit anti-human CD3 (Agilent, Santa Clara, USA) antibodies. Bound primary antibodies were detected using the EnVision detection system (Agilent, Santa Clara, USA). The reaction was visualised with DAB (Fluka). The samples were subsequently counterstained with Gill's haematoxylin. Immunohistochemically, the lymphoblasts showed positivity for CD79α (Figure 3D).

Phenotypic characterisation of the mesenteric lymph node cells was performed using two-colour flow cytometry (FC) (Faldyna et al. 2001). Cells were characterised based on forward and side scatter characteristics and by the expression of selected surface and intracellular molecules. The monoclonal antibodies used are summarised in Table 1.

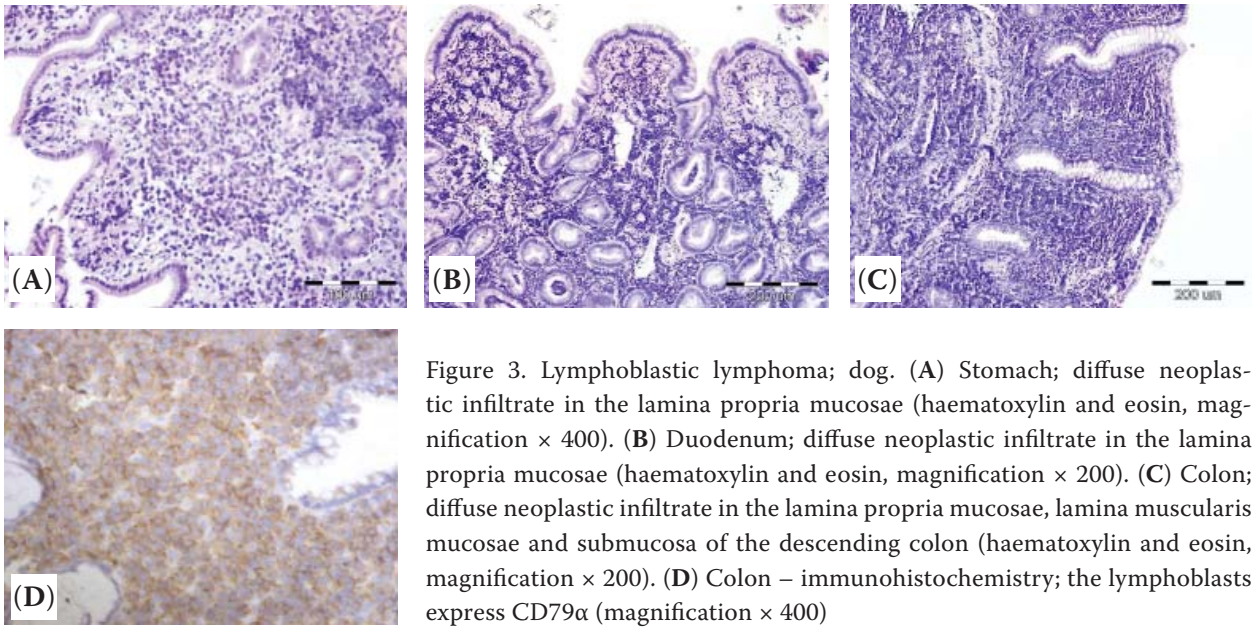

Figure 3. Lymphoblastic lymphoma; dog. (**A**) Stomach; diffuse neoplastic infiltrate in the lamina propria mucosae (haematoxylin and eosin, magnification × 400). (**B**) Duodenum; diffuse neoplastic infiltrate in the lamina propria mucosae (haematoxylin and eosin, magnification × 200). (**C**) Colon; diffuse neoplastic infiltrate in the lamina propria mucosae, lamina muscularis mucosae and submucosa of the descending colon (haematoxylin and eosin, magnification × 200). (**D**) Colon – immunohistochemistry; the lymphoblasts express CD79α (magnification × 400)

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Table 1. Monoclonal antibodies used in the study

Monoclonal antibody	Specificity	Expression	Source
CA17.2A12*	CD3	T lymphocytes	P.F. Moore, U.C. Davis, USA
CA13.1E4*	CD4	T helpers	P.F. Moore, U.C. Davis, USA
CA9.JD3*	CD8	T cytotoxic	P.F. Moore, U.C. Davis, USA
CA20.8H1*	γδ-TCR	γδ-T lymphocytes	P.F. Moore, U.C. Davis, USA
HM57#	CD79α	B lymphocytes	Agilent, Santa Clara, USA
CA2.1D6*	CD 21	mature B lymphocytes	P.F. Moore, U.C. Davis, USA
CA1.4G8*	CD90	T lymphocytes, many others	P.F. Moore, U.C. Davis, USA
CA2.1C12*	MHC-II	many cells	P.F. Moore, U.C. Davis, USA
TŰK4#	CD14	monocytes	Agilent, Santa Clara, USA
CA12.10C12*	CD45	leukocytes	P.F. Moore, U.C. Davis, USA

*canine-specific

#human cross-reactive

Cells were stained using the indirect immunofluorescence technique as described elsewhere (Faldyna et al. 2005). Intracellular staining of CD79α was performed after fixation and permeabilisation using the IntraStain kit (Agilent, Santa Clara, USA) in accordance with the manufacturer's recommendations. Control samples were stained with secondary antibodies only. Data were acquired on a standard FACSCalibur™ flow cytometer (Becton Dickinson, Mountain View, USA), operated by the CELLQuest™ software.

Flow cytometry revealed two populations of lymphocytes. Both expressed the pan-leukocyte marker CD45 and did not express the monocyte marker CD14 (not shown). Larger (malignant) lymphocytes did not express the T-associated markers CD3, CD4, CD8, γδ-TCR and CD90. However, they did express B-specific antigen CD79α, but not CD21 (Figure 4). The B-lymphocyte phenotype was further confirmed by the expression of CD45RA (isoform of CD45 expressed on all B-lymphocytes and naive T-lymphocytes) and MHC II. Smaller (healthy) lymphocytes expressed both T and B-lymphocyte markers. When compared to expected values, a higher percentage of CD8-positive lymphocytes was detected (Faldyna et al. 2005). No lymphoma cells were detected in the peripheral blood by morphological examination or FC analysis.

The dog was diagnosed with gastrointestinal B-lymphoblastic lymphoma (B-LBL), clinical stage V (substage b) and was treated with a six-month modified version of the University of Wisconsin-Madison protocol for malignant lymphoma in dogs (Garrett et al. 2002). This protocol comprises vincristine, cyclophosphamide, doxorubicin, prednisone and L-asparaginase. L-asparaginase was not administered and was reserved for the rescue therapy. L-carnitine, coenzyme Q10 and n-acetylcysteine were given before doxorubicin administration; n-acetylcysteine was also given before cyclophosphamide administration. Dietary supplementation with n-3 polyunsaturated fatty acids, inositol hexaphosphate and β-glucan was recommended.

The patient was doing well on chemotherapy, with no apparent toxicity. At the owner's request, routine control endoscopy was not performed after completing the induction phase of the protocol. The dog finished the 25-week chemotherapy protocol and remained completely asymptomatic during the first complete clinical remission for 395 days until he relapsed. He was presented with poor body condition, vomiting and diarrhoea. Marked abdominal lymphadenopathy was detected. Cytological examination of the abdominal lymph nodes was performed and confirmed the relapse (Figure 5). Reinduction was attempted with the original protocol. The dog achieved a second complete clinical remission but relapsed again at week nine of the protocol. The combination of lomustine, prednisone and L-asparaginase was recommended as a rescue therapy. The dog was hospitalised and supportive treatment consisted of intravenous fluids, amoxicillin-clavulanate, famotidine and metoclopramide at the same doses as previously and prednisone 2 mg/kg body weight, *p.o. q* 24 h. L-asparaginase was administered at a dose of 10.000 IU/m^2 intra-

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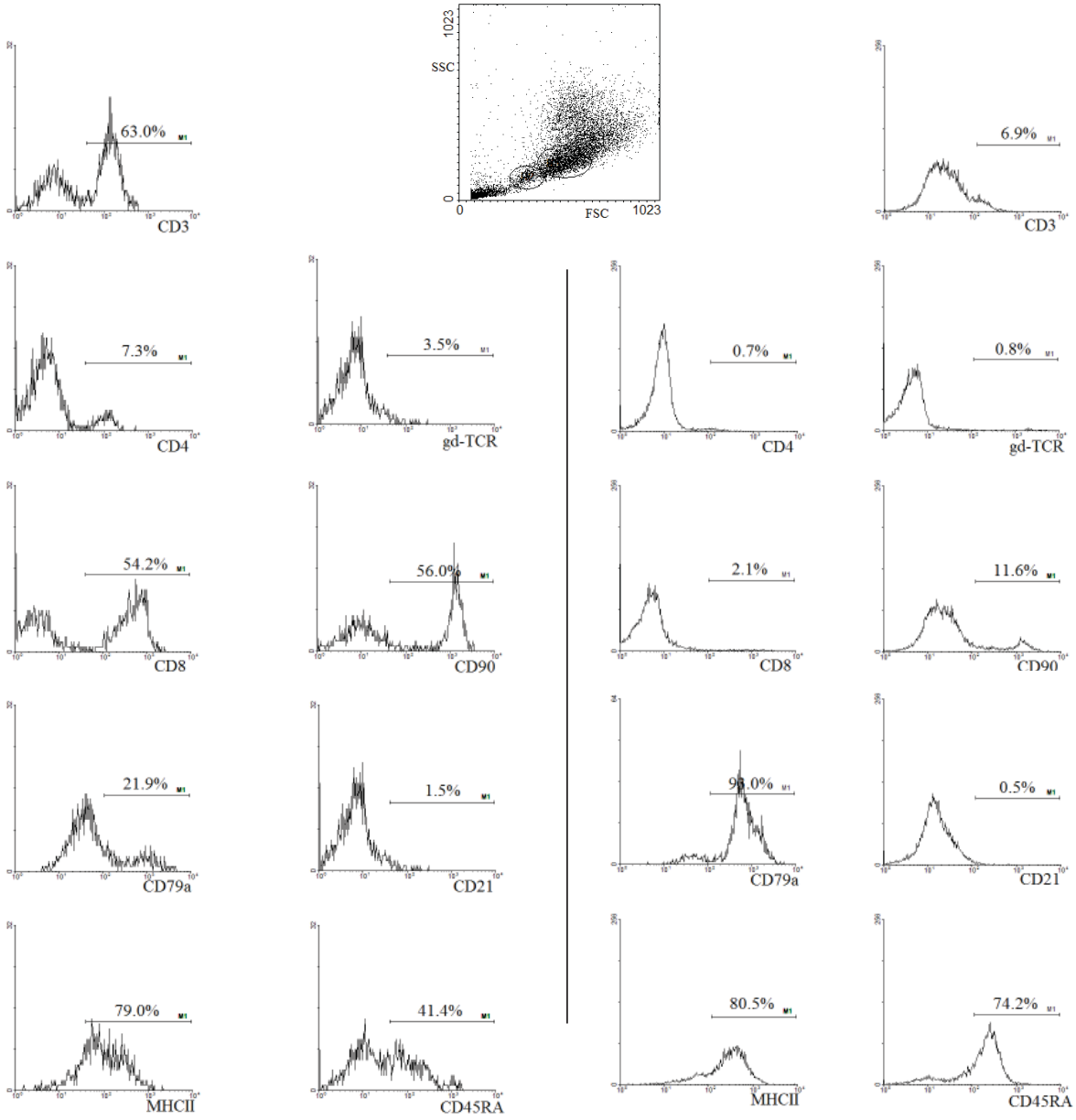

Figure 4. Results of a flow cytometric analysis of a sample from the mesenteric lymph node. Lymphocytes and blasts can be distinguished on the basis of forward and side scatter characteristics. The presence of intracellular and cell surface antigens on lymphocytes (left) and blasts (right) are shown in the respective histograms

muscularly and the patient was monitored for any hypersensitivity reaction. Lomustine treatment was scheduled three days later. The dog achieved partial remission and was eating and drinking, but was euthanised at the owner's request due to marked weakness. Progression-free interval was 382 days and the overall survival time was 487 days.

Additionally, PCR for antigen receptor rearrangement (PARR) was performed (USA). For PARR testing, impression smears with endoscopically-derived samples from the stomach and duodenum were used (samples taken at the time of diagnosis), but the samples were non-diagnostic. Endoscopically-derived samples from the ileum (obtained at the time of diagnosis) and FNAB specimen of mesenteric lymph node (relapse sample) were also used (Laboklin). DNA was extracted using the MagNA Pure 96 DNA Kit (Roche) according to the manufacturer's instructions. PARR was performed via GeneScan using the Genetic Analyser 3130 (Applied Biosystems). Primers were used according to Gentilini et al. (2009) for B-cells and Keller

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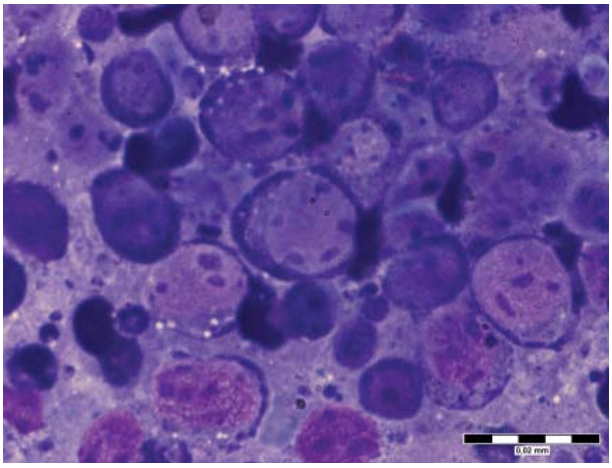

Figure 5. Cytological examination. Fine-needle aspiration biopsy of mesenteric lymph node; medium-sized lymphocytes with a thin rim of deeply basophilic cytoplasm. The nuclei are round or slightly irregular in shape, with finely stippled chromatin and multiple nucleoli (Dip Quick Stain; magnification × 1000)

and Moore (2012) for T-cells. IgH monoclonality was confirmed both in samples taken at the time of diagnosis and relapse.

DISCUSSION AND CONCLUSIONS

We demonstrated an unusual case of primary gastrointestinal B-lymphoblastic lymphoma in a four-year old Bullmastiff with unusually long survival following chemotherapy and a good quality of life.

The Bullmastiff is a breed that has been identified as having a high incidence of lymphoma and cancer in general. Familial non-Hodgkin's lymphoma has been reported which suggests genetic predisposition (Onions 1984). The p53 mutation (even germ line) has been detected in some Bullmastiffs (Veldhoen et al. 1998) and mutation of this tumour suppressor gene predisposes individuals to cancer. However, the mutation status was not evaluated in this patient.

In humans, chronic inflammation is a well-documented cause of gastrointestinal lymphoma (mucosa-associated lymphoid tissue lymphoma) (Sagaert et al. 2010). Lymphocytic-plasmacytic inflammation was detected adjacent to and distant from the lymphoma in the reported case. Therefore, lymphocytic-plasmacytic enteritis (LPE), as a chronic intestinal inflammation, may initiate malignant transformation of the GI mucosa as suggested by French et al. (1996), although this has not yet been proven in dogs.

The blood results in patients with alimentary lymphoma are non-specific, but often include neutrophilia, anaemia and hypoalbuminaemia (Couto et al. 1989; Miura et al. 2004; Frank et al. 2007). Mild hypoalbuminaemia that normalised within 14 days following therapy was identified in the reported dog. Both lymphoma and LPE could contribute to low serum albumin levels due to protein-losing enteropathy. Inhibition of albumin synthesis in the liver by circulating cytokines has also been reported in lymphoma patients (Heinrich et al. 1990).

More than 40% of dogs with lymphoma are anaemic at the time of diagnosis, regardless of the anatomic form of the disease (Abbo and Lucroy 2007). Anaemia is typically normocytic normochromic. It was assumed in this case of microcytic hypochromic anaemia that pre-existing anaemia of iron deficiency and anaemia of inflammation could be present due to inflammatory bowel disease (Weiss and Gasche 2010) and that these conditions were subsequently worsened by lymphoma. Lymphoma patients without bone marrow infiltration (which was not examined) may have anaemia of inflammation due to excessive release of cytokines such as IL-6 (Tisi et al. 2014). Lucroy et al. (1998) suggested that the bone marrow responds inadequately to erythropoietin.

Abdominal ultrasound may reveal a markedly thickened hypoechoic gastric/intestinal wall, loss of intestinal layering, a hyperechoic luminal interface with acoustic shadowing and the absence of motility. Marolf et al. (2015) reported little agreement between sonography and endoscopy of the stomach, with sonography identifying only 50% and endoscopy identifying 95% of gastric neoplasms. No sonographic abnormalities are revealed in more than 25% of cases of gastrointestinal lymphoma (Frances et al. 2013). Our findings are in agreement with previously mentioned studies, as only changes in the colon and enlargement of abdominal lymph nodes were detected.

Ultrasound-guided FNAB can be used to sample enlarged abdominal lymph nodes. It is valuable in the evaluation of tumour distribution and can also be very useful if the intestinal lesion is inaccessible for endoscopic biopsy (Yam et al. 2002). In contrast, Lingard et al. (2009) reported that almost 90% of fine-needle aspirates in cats with abdominal

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lymphadenopathy were incorrectly diagnosed as benign hyperplasia, but reclassified as malignant based on histological examination. According to Bonfanti et al. (2006), the sensitivity and specificity of the cytological examination of fine-needle aspirates from the GI tract in dogs is 71 and 100%, respectively. For impression smears, sensitivity and specificity are reported to be 100%.

Obtaining tissue samples is essential for diagnosing lymphoma. Flexible endoscopy, laparoscopy and laparotomy are three potential options. Endoscopy, which was chosen in the reported dog, is the least invasive technique; it allows inspection of gastric and intestinal mucosa and the collection of multiple samples from different sites (Washabau et al. 2010). According to Willard et al. (2008), endoscopy can be an effective tool for diagnosing GI mucosal lymphoma in dogs. The samples should be obtained from all endoscopically accessible segments of the GI tract. Every effort must be made to obtain biopsy samples from as deep in the lamina propria as possible so that accurate differentiation is possible on histological examination (Tams and Webb 2011). On the other hand, full-thickness biopsy is still considered superior to endoscopic biopsy (Kleinschmidt et al. 2006). In this lymphoma patient, endoscopically obtained samples were sufficient to establish the diagnosis of lymphoma in stomach, duodenum, ileum and colon, despite the presence of concomitant LPE.

LPE is a common finding and complicates the diagnostics. It can be difficult to differentiate lymphoma from LPE based on morphology alone, and special tests that enhance the accuracy of histopathology are needed (Evans et al. 2006; Carrasco et al. 2015).

Histopathology is not only important for confirmation of the cytological diagnosis, it is essential for the lymphoma subtype identification. Primary gastrointestinal B-LBL is rarely diagnosed in veterinary (Ponce et al. 2010) and human medicine (Raderer and deBoer 2010). Although alimentary lymphomas accounted for only 1.48% of the total of 608 dogs, no precursor B-cell lymphomas were diagnosed by Ponce et al. (2010). All B lymphomas exhibited the mature B-cell phenotype. Ohmura et al. (2017) diagnosed one case of B-LBL out of 29 dogs with GI lymphoma. Sozmen et al. (2005) reported an 8% prevalence of B-LBL; however, all dogs showed the multicentric form. It is difficult to evaluate which is the most common morphological subtype in this anatomic location as the data is usually missing.

Immunophenotyping increases the accuracy of diagnostics and sub-classification of most lymphoma subtypes (Comazzi and Gelain 2011). The immunophenotype is also a well-established prognostic marker for canine lymphoma (Dobson et al. 2001), and FC is a suitable method for immunophenotypic analysis (Borska et al. 2009).

In contrast to humans (Sagaert et al. 2012), most cases of primary GI lymphomas in dogs are of T-cell origin (Couto et al. 1989; Coyle and Steinberg 2004). Carrasco et al. (2015) reported a 100% prevalence of T-cell alimentary lymphomas, but the samples were taken from duodenum only. No patient affected by gastric or colorectal lymphoma was included in the study. B-cell phenotype typically predominates in these locations (Frank et al. 2007; Van den Steen et al. 2012).

B lymphomas account for approximately 15–37% of GI lymphomas (Frank et al. 2007; Ohmura et al. 2017). In the reported patient, lymphoblasts from mesenteric lymph node expressed the pan-B marker CD79α. As they did not express the marker of mature B-lymphocytes CD21, it was concluded that the malignant cells were immature B-lymphocytes (Faldyna et al. 2003). This differentiates lymphoblastic lymphoma (precursor B-cell neoplasm) from other (mature) histological subtypes.

Clonality testing is a useful adjunctive diagnostic test to standard histopathology (Thalheim et al. 2013). In comparison with FC, the sensitivity of PARR in determining the correct diagnosis is significantly lower (67% versus 91%) (Thalheim et al. 2013). According to Fukushima el al. (2009), the sensitivity of PARR is lower (< 70%) in endoscopically-derived samples from the GI tract than for other lymphoid neoplasms in dogs. This is especially true in patients with concomitant LPE, as inflammatory lymphocyte infiltration decreases the number of tumour cells.

Cytological specimens with impression smears of endoscopically-derived samples from the stomach were inadequate for determining the clonality, probably due to the low content of DNA as reported by Kaneko et al. (2009). On the other hand, endoscopically-derived samples and FNAB samples were sufficient for PARR testing in another laboratory and IgH monoclonality was confirmed. Flow cytometry and immunohistochemistry verified the B-cell phenotype of the malignancy.

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To distinguish alimentary lymphoma from inflammatory bowel disease, Carrasco et al. (2015) recommend combining histopathology, immunophenotyping, determination of the Ki-67 index and clonality testing.

The current report is limited due to the fact that immunohistochemistry and PARR were not performed in all biopsy samples. This would support a B-cell origin of the lymphoma in all endoscopically accessible parts of the GI tract, especially in the duodenum. Unfortunately, histological blocks were unavailable at the time of writing. We do not believe that two different lymphomas were present in this case, but rather suggest that the B-cell lymphoma exhibited a diffuse character.

Chemotherapy has been reported to be ineffective in most patients with GI lymphoma. Despite treatment, patients are usually euthanised within a few weeks due to a worsening of their clinical status (Couto et al. 1989; Frank et al. 2007). However, CHOP-based protocols have resulted in durable remissions in some cases (Frank et al. 2007; Rassnick et al. 2009).

Diarrhoea at the time of presentation and no response to chemotherapy are associated with short survival time according to Rassnick et al. (2009).

Although hypoalbuminaemia is associated with shorter remission duration in dogs with the multicentric form of lymphoma, the effect on remission duration and survival in the alimentary form of lymphoma is unclear (Frank et al. 2007).

Anaemia was confirmed to be another prognostic marker in dogs with the multicentric form of lymphoma (Marconato el al. 2011). Abbo and Lucroy (2007) suggest that anaemic dogs are four times less likely to achieve a complete remission following chemotherapy. Dogs with anaemia had a significantly shorter median survival time, but the anatomic location was not determined. Despite anaemia and hypoalbuminaemia at the time of presentation, a long survival was achieved in the reported dog.

A study of Valli et al. (2013) suggests that achieving remission can be very difficult in lymphoblastic lymphoma, regardless of anatomic location. In spite of this data, the patient achieved a complete clinical remission, he tolerated chemotherapy well and there was no evidence of GI toxicity.

The median overall survival time is 77 days for dogs with GI lymphoma and 106 days for dogs with B-cell GI lymphoma (Rassnick et al. 2009). Median survival time is longer for dogs with B-cell GI lymphoma in comparison to those with the T-cell lymphoma, but the difference is not statistically significant (Rassnick et al. 2009). Marconato et al. (2011) noticed higher complete response rates among dogs with B-cell lymphomas treated with a CHOP-based protocol.

Lymphomas in the colorectal location seem to have a more favourable prognosis (Frank et al. 2007; Van den Steen 2012).

The overall survival time (487 days) exceeded the longest survival time of a dog with primary GI B-cell lymphoma reported by Rassnick et al. (2009) and this despite the lymphoma being histologically confirmed in the stomach, small and large intestine. Despite the negative prognostic indicators, complete clinical remission and long survival was achieved.

With respect to the poor prognosis and aggressive clinical course of the disease, we suggest that the use of an aggressive multi-agent chemotherapy protocol in dogs with alimentary B-LBL may provide a good quality of life with survival equivalent to that observed in the multicentric form. Further studies are required to confirm this idea.

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