Canine lymphoma: a retrospective study (2009 – 2010)

Canien lymfoom: een retrospectieve studie (2009 – 2010)

F. Mortier, S. Daminet, S. Vandenabeele, I. Van de Maele

Department of Small Animal Medicine and Clinical Biology
Faculty of Veterinary Medicine, Ghent University
Salisburylaan 133, B-9820 Merelbeke, Belgium

femkemortier@hotmail.com

ABSTRACT

This study reviews the medical records of 56 dogs diagnosed with lymphoma based on the cytological and/or histological results between January 1, 2009 and December 31, 2010. Most of the dogs were middle-aged to old, and were diagnosed with multicentric lymphoma (ML) (n=36). The majority of the dogs were presented in stages III to V (n=55) and substage b (n=43). A complete blood count and serum biochemistry, urinalysis, serum protein electrophoresis, thoracic radiographs and/or abdominal ultrasound were performed. The results correlated with previously described results in the literature. Therapy was initiated in 80% of the dogs (n=45). After diagnosis, the median survival time of 62% of these dogs (n=28) treated with only prednisolone was 32 days (range 3 – 224 days). For 24% of the dogs (n=11) treated with chemotherapy, the median survival time was 119 days (range 11 - 273 days). Surgical resection of the macroscopic tumor was performed in the remaining six dogs (13%). Three of these dogs received subsequent prednisolone therapy. The median survival time of these six dogs was 47 days (range 0 – 669 days). The dogs that received chemotherapy had significantly longer survival times than those treated with only prednisolone, although negative prognostic factors were present in all of the cases treated with chemotherapy.

SAMENVATTING

Het medisch dossier van 56 honden met een cytologische en/of histologische diagnose van lymfoom gesteld tussen 1 januari 2009 en 31 december 2010 werd bestudeerd. De meeste honden waren van middelbare leeftijd of ouder en hadden multicentrisch lymfoom (n=36). De meerderheid van de honden bevond zich in stage III tot V (n=55) en in substage b (n=43). Hematologie, serumbiochemie, urineanalyse, serumeiwit-elektroforese, thoraxradiografieën en/of abdominale echografie werden uitgevoerd en de resultaten kwamen grotendeels overeen met deze in de literatuur. Bij 80% (n=45) werd een behandeling opgestart. Na de diagnose was de mediane overlevingstijd voor 62% van de honden (n=28) die enkel met prednisolone werd behandeld, 32 dagen (3 – 224 dagen). Voor 24% (n=11) behandeld met chemotherapie was dit 119 dagen (11 – 273 dagen). Bij de resterende zes behandelde honden (13%) werd de macroscopische tumor verwijderd middels chirurgie. Drie van deze honden werden nadien behandeld met prednisolone. De mediane overlevingstijd van deze zes honden bedroeg 47 dagen (0 – 669 dagen). Van de honden die met chemotherapie werden behandeld, was de mediane overlevingstijd significant langer dan van de honden die enkel met prednisolone werden behandeld, ondanks het feit dat negatief prognostische factoren aanwezig waren bij alle honden die chemotherapie ondergingen.

INTRODUCTION

Lymphoma, also referred to as lymphosarcoma (LSA) or malignant lymphoma, is one of the most prevalent neoplasms in the dog. It accounts for 7% to 24% of all canine tumors and up to 83% of all canine hematopoietic malignancies (Vail and Young, 2007; Vail, 2010). LSA arises due to the proliferation of malignant lymphoid cells – usually in lymphoid tissue, such as lymph nodes, liver or spleen, but the tumor may originate in practically any tissue (Vail and Young, 2007; Couto, 2009; Vail, 2010). This origin from solid organs distinguishes LSA from lymphoid leukemia, as the latter arises from bone marrow (Couto, 2009). The etiology of LSA is considered to be multifactorial. Several environmental, infectious, immune-mediated and genetic factors are associated with a higher risk of developing LSA (Keller, 1992; Gavazza et al., 2001; Blackwood et al., 2004; Farinha and Gascoyne, 2005; Modiano et al., 2005; Brunker and Hoover, 2007; Santoro et al., 2007; Huang et al., 2012). Affected animals are mainly middle-aged to older dogs, and there is no sex predilection (Parodi et al., 1968).

There are four different anatomical forms: multicentric (characterized by generalized lymphadenopathy (GLA)), alimentary (characterized by infiltration

Table 1. WHO clinical staging for domestic animals with LSA (Vail and Young, 2007; Vail, 2010).

Stage	Criteria
I	Single lymph node involved (or lymphoid tissue in one single organ)
II	Multiple lymph nodes involved in a regional area
III	Generalized lymph node involvement
IV	Liver and/or spleen involved (+/- stage III)
V	Bone marrow, blood and/or nonlymphoid organs involved (+/- stage I to IV)

Substage a = Without clinical signs of disease
Substage b = With clinical signs of disease

of the gastrointestinal tract), mediastinal and extranodal (affecting any other organ or tissue). Multicentric lymphoma (ML) is the most common form in dogs – accounting for more than 80% of all canine lymphomas –, and is a clinically aggressive neoplasm comparable to high-grade non-Hodgkin's lymphoma in humans (Couto, 2009; Marconato, 2011). The clinical findings are strongly related to the anatomical form. Although clinical signs and physical examination are often suggestive of LSA, a definitive diagnosis requires cytology, histopathology or molecular diagnostics (Couto, 2009). Once the diagnosis is confirmed, stage and substage are determined according to the World Health Organization (WHO) classification system reproduced in Table 1.

Treatment of LSA with multi-agent chemotherapy protocols is initially gratifying, with response rates up to 90% (Vail, 2010). Equally important, most owners feel that the animal's quality of life during treatment is good, and do not regret pursuing palliative chemotherapy (Mellanby et al., 2003; Bergmann et al., 2011). The prognosis varies, and it is negatively influenced by several factors, such as WHO stage V and substage b, mediastinal location, intermediate or high histological grade, T-cell type, previous treatment with corticosteroids or chemotherapy and the presence of hypercalcemia. Eventually, most animals die after relapse of chemotherapy-resistant, disseminated disease (Vail and Young, 2007; Vail, 2010; Marconato et al., 2011).

The aim of this retrospective study is to review the medical records and to describe the signalment, clinical signs, physical examination findings, laboratory and medical imaging abnormalities, the anatomical form, stage and substage, the therapy used and the overall survival time of dogs with LSA at the Department of Small Animal Medicine and Clinical Biology, Faculty of Veterinary Medicine, Ghent University.

MATERIALS AND METHODS

Criteria for the selection of the cases

Retrospectively, the medical records of 72 dogs admitted to the Department of Small Animal Medicine and Clinical Biology of the Faculty of Veterinary Medicine (Ghent University), between January 1, 2009 and December 31, 2010 with a presumptive diagnosis of LSA were reviewed in detail. A cytological or histological diagnosis of LSA was required to be included in the study. For a cytological diagnosis of LSA, one of the following criteria had to be met: lymphoblasts accounting for > 50% of all nucleated cells present in a lymph node (Cowell et al., 2003), the presence of ≥ 30% lymphoblasts and lymphocytes in bone marrow (Grindem et al., 2002) or the presence of a monomorphic population of lymphoblasts in non-lymphoid tissue (Webb et al., 2008). Dogs with a confirmed diagnosis of acute lymphoblastic leukemia or dogs whose medical record was incomplete were excluded. Fifty-six dogs met the inclusion criteria.

Procedures

The medical records were reviewed for information collected at the first examination including signalment, clinical signs, physical examination findings, blood examination results (complete blood count (CBC), serum biochemical profile, coagulation profile and serum protein electrophoresis), results of urinalysis, medical imaging, cytology, histopathology and immunophenotyping. The WHO clinical staging system for LSA in domestic animals was used to determine stage and substage in all of the 56 cases. Treatment, survival time after diagnosis, response to therapy and adverse events associated with treatment were reviewed for the 20 dogs followed up at the clinic. The response to chemotherapy was classified by the use of the following categories: complete remission (CR: 100% reduction of all measurable lesions), partial remission (PR: ≥ 50% but <100% reduction in size of all measurable lesions), stable disease (SD: < 50% reduction in size of all measurable lesions or no change and no new neoplastic lesions), and progressive disease (PD: > 25% increase in size or the appearance of new neoplastic lesions) (Ettinger, 2003). Hematologic and gastrointestinal toxicosis were assessed based on the medical records, applying the common terminology criteria for adverse events (CTCAE) (Veterinary cooperative oncology group, 2004). Telephonic contact with the referring veterinarian and/or the owner provided additional information regarding survival time in another 34 of the cases. Two dogs were lost to follow-up.

Statistical analysis

The data was interpreted by means of descriptive statistics. The results are expressed as mean ± SD with range. Because some of the clinicopathological tests

were carried out at different laboratories with different reference ranges, only the percentages of the dogs having values above, within or beneath the reference range are provided.

RESULTS

Signalment

The mean age at presentation was 7.6 years ± 3.3 years (range, 1.7 to 14.4 years). The mean body weight was 30.5 kg ± 14.1 kg (range, 4.4 to 64.5 kg). Eighteen dogs (32.1%) were male intact, 7 (12.5%) male neutered, 12 (21.4%) female intact and 19 (33.9%) female spayed. Twenty-nine breeds were represented in the study. The breed distribution is shown in Table 2.

History, clinical signs and physical examination findings

The most common clinical signs and physical examination findings at presentation are shown in Figure 1. The duration of the clinical signs at the time of presentation varied from 1 to 135 days, with a median duration of 22 days. Twenty-five (44.6%) dogs had previously been treated with corticosteroids by the referring veterinarian, and one dog (1.7%) had previously been treated with a multi-agent chemotherapy protocol (doxorubicin, cyclophosphamide and prednisolone).

Table 2. Prevalence of dog breeds diagnosed with LSA.

Breed	Total	%
Labrador retriever	9	16.1
Mixed breed	8	14.3
Bernese mountain dog, Bouvier de Flandres, Golden retriever	3 each	5.4
American Staffordshire terrier, Border collie, Boxer, Flatcoated retriever, German shepherd dog, Rottweiler	2 each	3.6
Beagle, Bullmastiff, Canadian white shepherd, Chihuahua, Dutch kooiker hound, English bulldog, English cocker spaniel, German shorthaired pointer, Great Dane, Griffon korthals, Maltese, Rough collie, Saint Bernard, Shar-pei, Standard Schnauzer, Dachshund, Tibetan terrier, Welsh corgi	1 each	1.8

Table 3. Results of CBC, serum biochemistry and coagulation, plus the percentages of normal, elevated and decreased results.

	Number of dogs tested	Normal (% of the dogs tested)	Elevated (% of the dogs tested)	Decreased (% of the dogs tested)
Hematology				
Red blood cells	n=42	26 (61.9%)	1 (2.4%)	15 (35.7%)
White blood cells	n=40	19 (47.5%)	18 (45%)	3 (7.5%)
► Lymphocytes	n=22	14 (63.6%)	7 (31.8%)	1 (4.5%)
► Neutrophils	n=22	9 (40.9%)	12 (54.5%)	1 (4.5%)
Thrombocytes	n=40	22 (55%)	1 (2.5%)	17 (42.5%)
Biochemistry				
Urea	n=43	34 (79.1%)	8 (18.6%)	1 (2.3%)
Creatinine	n=43	37 (86%)	6 (14%)	0
Liver enzymes	n=43	20 (46.5%)	22 (51.2%)	1 (2.3%)
Total bilirubin	n=9	6 (66.6%)	3 (33.3%)	0
Total protein	n=43	28 (65.1%)	4 (9.3%)	11 (25.6%)
Albumin	n=43	30 (69.8%)	2 (4.7%)	11 (25.6%)
Calcium	n=24	19 (79.2%)	5 (20.8%)	0
Coagulation				
PT[a]	n=9	8 (88.9%)	1 (11.1%)	0
aPTT[b]	n=9	8 (88.9%)	1 (11.1%)	0
D-dimers	n=8	3 (37.5%	5 (62.5%)	0
Fibrinogen	n=8	6 (75%)	2 (25%)	0

[a]Prothrombin time
[b]Activated partial thromboplastin time

Table 4. Most common medical imaging findings.

Thoracic radiograph findings (n=29)	**Number of dogs**	**% of dogs tested**
No abnormalities	13	**44.8**
Diffuse interstitial lung pattern	7	**24.1**
Alveolar lung pattern	5	**17.2**
Suprasternal or tracheobronchial LA*	5	**17.2**
Pleural effusion	5	**17.2**
Cranial mediastinal mass	3	**10.3**
Abdominal ultrasound findings (n=28)	**Number of dogs**	**% of dogs tested**
No abnormalities	4	**14.3**
Abnormal spleen (hypoechoic, splenomegaly, nodules)	15	**53.6**
Abdominal LA	14	**50**
Abnormal liver (hypoechoic, hepatomegaly)	9	**32.1**
Gastrointestinal wall thickening and/or loss of layering	7	**25**
Ascites	3	**10.7**
Abdominal mass of unknown origin	3	**10.7**

* LA: lymphadenopathy

Clinicopathologic findings

The blood examination results are described in Table 3. In nine cases (16.1%), no blood examination was performed. Seven of these dogs suffered from ML and presented with generalized lymphadenopathy (GLA). The cytological examination of fine needle aspirations (FNA) of the peripheral lymph nodes was diagnostic, after which the dogs were euthanized (n=3), or palliative treatment with corticosteroids was started (n=4). The two other cases, in which no blood examination was performed, were a dog with central nervous system LSA that was euthanized immediately after diagnosis and a dog with laryngeal LSA that was treated with corticosteroids.

Serum protein electrophoresis was performed in six dogs (10.7%) and showed a monoclonal gammopathy in two of them (33.3%). Tests for tick-borne diseases were performed in four of the dogs (7.1%), and all results were negative.

The urine of 15 dogs (26.8%) was analysed. Isosthenuria ($1.007 \leq SG \leq 1.015$) was present in six (40%) and hypersthenuria ($SG > 1.015$) in nine of the dogs (60%). The urine protein to creatinine ratio was increased (> 0.5) in four of the 15 dogs (26.7%).

Medical imaging

The most commonly detected abnormalities on thoracic radiographs and abdominal ultrasound are presented in Table 4.

Cytology, histopathology and molecular diagnostics

The diagnosis of LSA was based upon the cytological results in 44 of the dogs (78.6%). Fine needle aspirates of peripheral lymph nodes were diagnostic for LSA in 33 of these 44 cases (75%). The cytological results of abdominal lymph nodes provided a definitive diagnosis of LSA in four cases (9.1%). Extranodal sites or fluids that were examined microscopically to confirm the diagnosis or to determine the clinical stage were the spleen (n=5; 11.4%), the liver (n=4; 9.1%), an abdominal mass of unknown origin (n=3; 6.8%), the kidney (n=2; 4.5%), the stomach, the small intestines, a cranial mediastinal mass, a laryngeal mass, pleural effusion and cerebrospinal fluid (1 each, 2.3%). Bone marrow aspirations were performed in five dogs and cytology revealed lymphoma in two dogs.

Histopathology provided the diagnosis in fourteen of the dogs included in this study (25%). The tissues examined were mucocutaneous lesions (n=5; 35.7%), peripheral lymph nodes (n=3; 21.4%), abdominal lymph nodes (n=2; 14.3%) and splenic nodules (n=2; 14.3%). Biopsies of stomach, small intestines and larynx were diagnostic in one dog each (7.1%). In two of the dogs (14.3%), the histopathological results indicated low-grade or indolent LSA. One of these dogs had splenic marginal zone LSA, and the other one had epitheliotropic lingual LSA.

In total, two dogs (3.6%) had both a cytological and a histological confirmation of LSA. In one of them, FNA of enlarged submandibular lymph nodes was diagnostic of LSA. In addition, biopsies of oral mucosal lesions were taken and revealed mucocutaneous LSA. The other dog with gastric perforation had FNA of abdominal lymph nodes, and partial gastrectomy was performed during emergency surgical exploration. Both the cytological results of the lymph nodes and the histological results of the gastric wall were diagnostic of LSA.

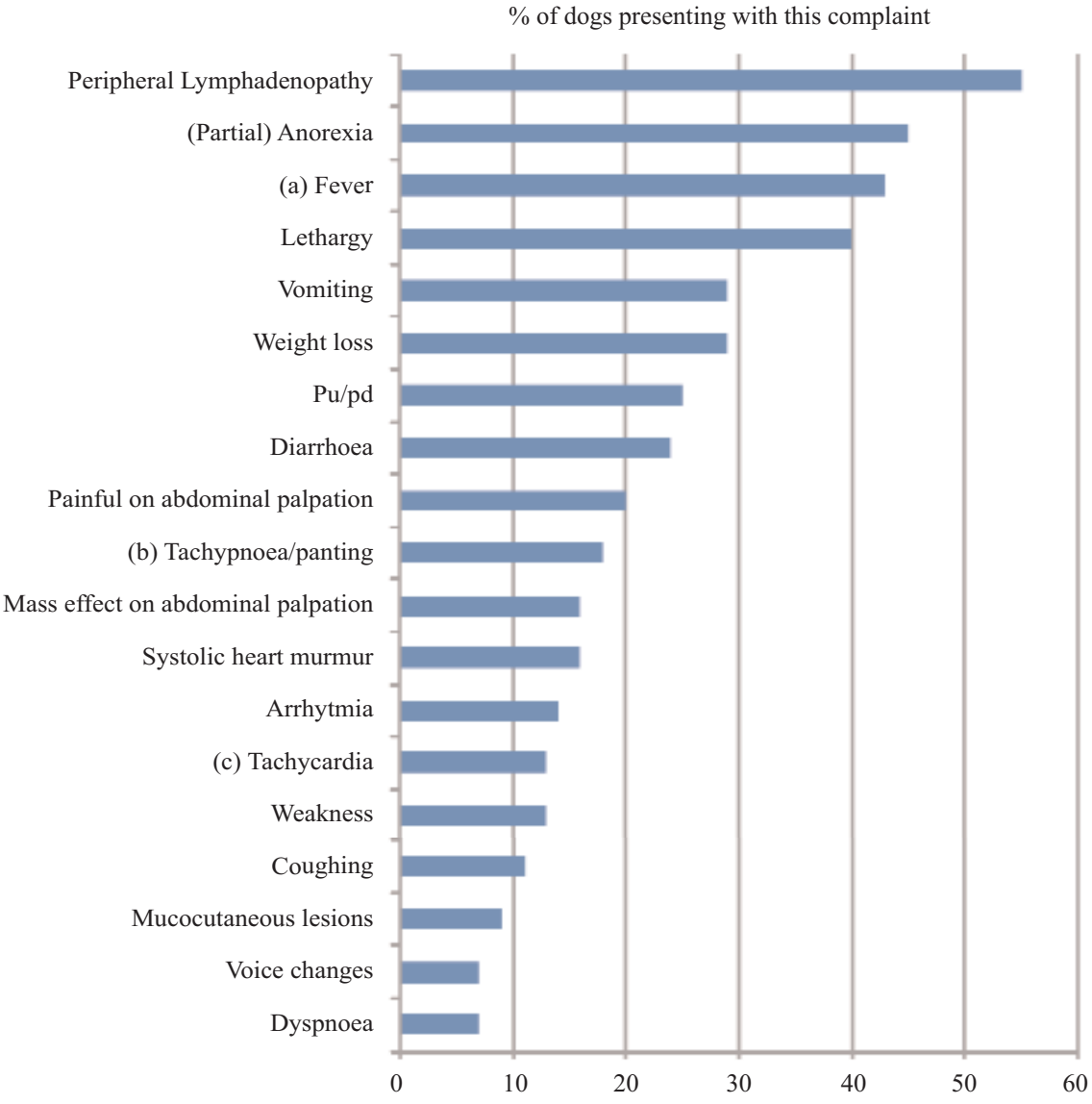
Immunophenotyping was performed in 17 dogs (30.4%), either through immunocytochemistry, immunohistochemistry or flow cytometry. The T-cell type predominated (n=10; 58.8%). In the remaining dogs,

Table 5. Treatment options and correlated survival times.

	Number of dogs	MST[a] (days)	Range (days)	Lost to follow-up	CR[b]	PR[c]
General outcome	56 (100%)	31	0-383	2		
Euthanasia[d]	11 (19.6%)	-	-	-		
Prednisolone only	28 (50%)	32	3-224	1		
Surgery	6 (10.7%)	47	0-669	1		
Chemotherapy	11 (19.6%)	119	11-273	-		
► L-VCA-Short	6 (10.7%)	109	11-273	-	2 (33.3%)	2 (33.3%)
► Single-agent	5 (8.9%) 31	129	69-272	-	3 (60%)	1 (20%)

[a] Median survival time
[b] Complete remission
[c] Partial remission
[d] Euthanasia or death before specific treatment could be initiated

Figure 1. Clinical complaints and most common physical examination findings at presentation of 56 dogs with LSA.



(a) Temperature > 39.2°C
(b) Respiratory rate > 40/minute
(c) Heart rate > 140/minute

LSA was B-cell type (n=7; 41.2%). Null-cell type LSA was not represented in the study.

Anatomical form, stage and substage

The distribution of the study population concerning anatomical location, stage and substage is shown in Figures 2, 3 and 4.

Concurrent diseases

Other diseases possibly related to LSA that were diagnosed at the time of presentation or that developed shortly thereafter, were disseminated intravascular coagulation, micro-angiopathic hemolytic anemia, immune-mediated hemolytic anemia, immune-mediated polyarthritis, chronic kidney disease (CKD), lymphocytic-plasmocytic enteritis (LPE) and endocarditis, each found in two dogs (3.6%). The source of endocarditis in one of these dogs was most likely an infection of the oral cavity that occurred secondary to the lesions caused by mucocutaneous LSA. One dog (1.8%) with intestinal LSA presented with an intestinal intussusception, and one dog (1.8%) with gastric LSA suffered from a septic peritonitis secondary to a ruptured gastric ulcer. One dog (1.8%) with stage V ML and severe thrombocytopenia (24,000 platelets/µl) had a pulmonary bleeding. Another dog (1.8%) with ML suffered from inflammation of muscle and fat tissue in the lumbosacral epidural area. Bilateral laryngeal paralysis and uveitis were seen in one dog each (1.8%).

Treatment and outcome

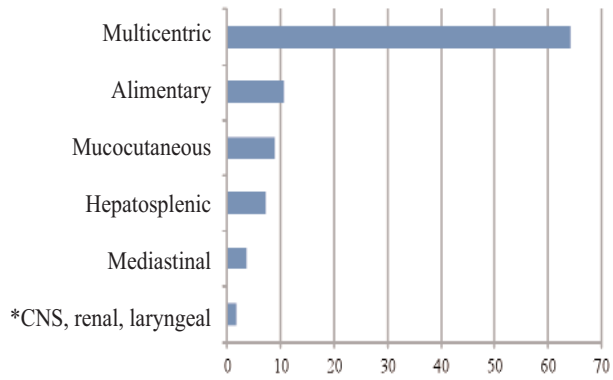
The median survival time (MST) and range for each treatment option applied to the dogs of the study are shown in Table 5.

Surgery was performed in six dogs: three of them with splenic LSA underwent splenectomy, two of the dogs with mucocutaneous LSA had nodulectomy, and in one dog with gastric LSA, partial gastrectomy was performed. Three of the surgically treated dogs were subsequently treated with prednisolone. One of the dogs with lingual low-grade LSA was still alive 383 days after the nodulectomy, and one of the dogs with splenic low-grade LSA was still alive 669 days after the splenectomy.

The L-VCA-Short protocol shown in Table 6 was used in six of the eleven cases that underwent chemotherapy. Five of them suffered from ML and one from mediastinal LSA. The five remaining dogs were treated with single-agent chemotherapy. One of these dogs with ML was treated with doxorubicin (30 mg/m^2 IV every 3 weeks), one dog with ML stage V or leukemia (bone marrow examination was not performed) with chlorambucil (0.2 mg/kg/d PO) and the three remaining dogs (two of these dogs with mucocutaneous and one with intestinal LSA) with lomustine (66 - 74 mg/m^2 PO every three weeks).

The side effects in ten of the eleven dogs treated with chemotherapy were studied, and occurred in six of the dogs (60%). Sterile hemorrhagic cystitis (SHC) was seen in one dog receiving cyclophosphamide, and hepatopathy occurred in two cases (20%) (in one case after treatment with lomustine and in the other case after vincristine administration). Neutropenia was present during the course of chemotherapy in three of the ten dogs (30%). Neutropenia was classified as CTCAE grade 2 in two dogs (once after lomustine and once after doxorubicin treatment) and as grade 4 in one dog (after cyclophosphamide administration). Thrombocytopenia was present in one dog (10%; grade 3) after vincristine administration. Gastrointestinal toxicosis occurred in four dogs (40%; grade 1 in three of the dogs, grade 4 in one of the dogs). In all four cases, gastrointestinal complaints started within five days after the first vincristine administration of the L-VCA-Short protocol. In two of these dogs, one more episode of gastrointestinal toxicosis was seen during the course of chemotherapy treatment – in one case, after doxorubicin and in the other case, after cyclophosphamide administration.

Figure 2. Percentage of dogs presenting with each anatomical form.



* CNS: central nervous system

Figure 3. Stage at the time of presentation. Figure 4. Substage at the time of presentation.

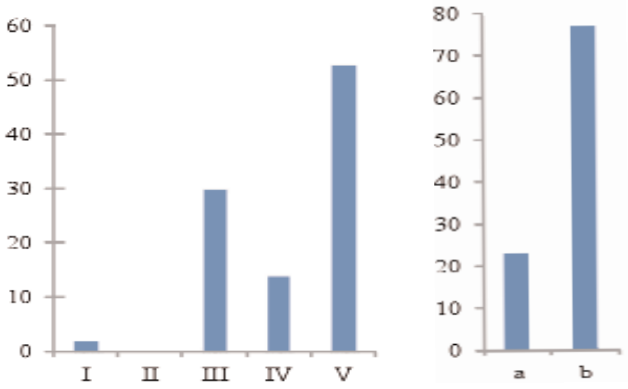


Table 6. L-VCA-Short protocol (Vail and Young, 2007).

Week	Drug	Dose	Week	Drug	Dose
1	Vincristine Prednisone	0.7 mg/m^2 IV 2 mg/kg/day PO	11	Vincristine	0.7 mg/m^2 IV
2	Cyclophosphamide Prednisone	250 mg/m^2 IV 1.5 mg/kg/day PO	12	Cyclophosphamide	250 mg/m^2 IV
3	Vincristine Prednisone	0.7 mg/m^2 IV 1 mg/kg/day PO	13	Vincristine	0.7 mg/m^2 IV
4	Doxorubicin Prednisone	30 mg/m^2 IV 0.5 mg/kg/day PO	14	Doxorubicin	30 mg/m^2 IV
5	No treatment		15	No treatment	
6	Vincristine	0.7 mg/m^2 IV	16	Vincristine	0.7 mg/m^2 IV
7	Cyclophosphamide	250 mg/m^2 IV	17	Cyclophosphamide	250 mg/m^2 IV
8	Vincristine	0.7 mg/m^2 IV	18	Vincristine	0.7 mg/m^2 IV
9	Doxorubicin	30 mg/m^2 IV	19	Doxorubicin	30 mg/m^2 IV
10	No treatment				

DISCUSSION

In the present study, the most commonly affected breed was the Labrador retriever, accounting for 16.1% of all the cases. However, this breed had previously not been identified to be at an increased risk for developing LSA. A possible explanation is the difference in breed popularity between different countries, with the Labrador retriever being a popular breed in Belgium.

The most frequent clinical signs observed in the present study were non-specific signs and/or a peripheral lymphadenopathy (LA). Other clinical signs and physical examination findings varied widely with the anatomical form present. In case of regional peripheral LA, the mandibular and prescapular lymph nodes were most commonly affectted. Polyuria and polydipsia (pu/pd) were observed in 26% of the dogs, and could be attributed to hypercalcemia (n=3), hepatopathy (n=4), previous treatment with corticosteroids (n=3) or furosemide (n=1) or CKD (n=2).

Hematologic and serum biochemical abnormalities may vary widely in dogs with LSA, and are not diagnostic (Couto, 2009). The anemia and thrombocytopenia found in a large percentage of the dogs of the present study were related to bone marrow infiltration, paraneoplastic immune-mediated destruction, splenic infiltration and/or chronic disease. Regenerative anemia may also be associated with concomitant blood loss (Couto, 2009; Vail, 2010). However, in one dog with bilateral renal LSA, polycythemia was present (hematocrit 72.8%, reference range 37%-55%). Polycythemia has been described in two dogs with renal T-cell LSA. The proposed pathogenesis is a combination of two mechanisms: the paraneoplastic production of erythropoietin by the tumor and an increased erythropoietin production by the remaining normal kidney cells, induced by local hypoxia due to compression of their vasculature (Durno et al., 2011). In dogs with leukocytosis, a differential cell count should be obtained to identify which leukocyte populations are involved. Lymphocytosis is known to occur in 20% of LSA cases and is usually of low magnitude (<10,000-12,000/µl) (Vail and Young, 2007; Couto, 2009). However, in the present study, lymphocytosis was present in seven dogs (31.8%) and the lymphocyte count exceeded 12,000/µl in four of them. These four dogs were classified as stage V ML cases, but leukemia could not be excluded with certainty in three of them, since bone marrow was only examined in one of these dogs. On serum biochemistry, the most common abnormalities are usually hypercalcemia and changes due to organ failure secondary to tumor infiltration (Couto, 2009, Vail, 2010). The increase in serum liver enzymes or bilirubin concentrations seen in over 50% of the dogs of the present study could be due to hepatic parenchyma infiltration and/or corticosteroid administration. The azotemia present in six dogs (16%) could be due to tumor infiltration, but also to dehydration, pre-existing CKD or hypercalcemic nephrosis (Vail, 2010).

Abdominal ultrasound is an important tool in the diagnosis and staging of LSA, as the presence of LSA can be confirmed with ultrasonographic guided FNA (Vail and Young, 2007). It was performed in 50% of the dogs of this study, more specifically in patients with gastrointestinal signs or palpable organomegaly on abdominal palpation or when serum biochemistry abnormalities were present, indicating possible organ involvement. It was not used as a stan-

dard staging procedure, as there is no prognostic difference between cases of ML with or without hepatic and/or splenic infiltration (i.e. stage IV or stage III ML) (Vail and Young, 2007; Vail, 2010).

Thoracic radiographs were performed in 50% of the dogs of the study population - either for diagnostic purposes (if the dog showed respiratory signs), or to determine the prognosis in case the owners were considering chemotherapy. This imaging modality is prognostically relevant, as there is a negative correlation between the presence of a mediastinal LA on the one hand and the remission duration and survival time on the other (Starrak et al., 1997). In a retrospective study of 84 dogs with ML, the most common abnormalities on thoracic radiographs were suprasternal LA (40%), pulmonary infiltration (37%), tracheobronchial LA (33%), cranial mediastinal LA (26%) and pleural change (23%). Pulmonary infiltration was suspected when a generalized interstitial pattern was present, most often reticular or micronodular (Blackwood et al. 1997). In the present study, possible pulmonary infiltration was present in 41.3% of the dogs in which radiographs were performed. However, the diffuse interstitial changes could have been related to age or chronic effects of air pollutants. It is also possible that dogs with ML and with unremarkable radiographs do have pulmonary involvement, which can be detected with bronchoalveolar lavage (Hawkins et al., 1993). Bronchoalveolar lavage was not performed in the study, and its correlation with prognosis is unknown.

Although clinical signs and physical examination are often suggestive of LSA – especially in cases of ML – a definitive diagnosis cannot be made without cytology, histology or molecular techniques. In 90% of the canine ML cases, FNA of peripheral lymph nodes will suffice. This is a minimally invasive and inexpensive technique for diagnosing LSA (Couto, 2009). In the present study, the cytological result of peripheral lymph nodes was conclusive in 33 of the 36 dogs with ML (91.7%). If the result of FNA of peripheral lymph nodes is inconclusive, biopsy of these lymph nodes should be performed. Histopathology of peripheral lymph nodes provided the diagnosis in three more dogs with ML. Preferably, the entire lymph node is removed (excisional biopsy) so the architecture of the node and the integrity of the capsule can be assessed (Vail and Young, 2007). If this is not an option, an incisional or Tru-cut biopsy can be performed (Ettinger, 2003).

Immunophenotyping is important as the T-cell phenotype correlates with a poorer prognosis in dogs with LSA (Vail and Young, 2007; Rebhun et al., 2010). Although B-cell LSA has been reported to account for 60% to 80% of all cases (Vail and Young, 2007), the T-cell phenotype predominated in the present study (58.8%). This can partially be explained by the fact that in the five cases of cutaneous LSA, biopsy and subsequent immunophenotyping were performed. This form of LSA is most classically represented by epitheliotropic T-cell LSA, also called mycosis fungoides (Bryan, 2010). These five dogs represent half of the cases of T-cell LSA of the present study.

Recently, the WHO classification, based on histological and immunophenotypically characteristics, has been applied to canine lymphomas (Valli et al., 2011). This classification system can aid in the future to better predict prognosis and therapeutic options.

The most common anatomical form in this study was ML, accounting for 64.3% of the cases. This percentage is less than the percentages described in the literature (i.e. 80% or more) (Couto, 2009; Vail and Young, 2010). The hypothesis is that the multicentric form is less frequently sent to a referral hospital than other forms of LSA. Additionally, 77.8% of the dogs with ML of the present study were classified as WHO substage b. This is in contrast with the 10% to 25% reported in previous articles (Keller *et al*., 1993; Vail *et al.*, 1996; Garrett *et al.*, 2002). This could be due to the fact that LSA is nowadays more readily detected and treated by practitioners, and especially more severe cases (i.e. clinically ill dogs) are referred to hospitals. The most frequently encountered extranodal form in the present study was mucocutaneous lymphoma. The type of lesions varied from depigmentation, erythema and desquamation to papules, plaques and nodules of the skin, mucocutaneous junctions and oral mucosa. It is important to realise that the cutaneous form of LSA may mimic practically any primary or secondary skin and mucosal lesion and is therefore called the "great imitator" (Couto, 2009).

In the present study, 98.2% of the dogs were presented in stage III, IV or V, which is more than the 80% reported in the literature (Vail and Young, 2007). The suspected reason for the large number of dogs presented at the Department of Small Animal Medicine and Clinical Biology, Faculty of Veterinary Medicine, Ghent University with late stage disease, is that it is a referral hospital. It should be noted that not every dog of the study was submitted to medical imaging or bone marrow examination. Therefore, underestimation of the clinical stage might have happened in some of the dogs with stage III disease. It is a well-known phenomenon that dogs are assigned a higher stage, as more sensitive staging methods have been introduced. This so-called stage migration makes it difficult to compare response to treatment between studies that apply different staging techniques. Ideally, a standard protocol should be developed to stage dogs with LSA (Flory *et al.*, 2007).

When left untreated, the expected survival time for dogs with LSA is four to six weeks (Vail, 2010). There are several different therapeutic strategies for LSA, but it is important for both the clinician and the owner to realise that in most cases, treatment of LSA is not curative. The main goals are to offer palliative treatment that improves the quality of life by diminishing the clinical signs and to prolong the lifespan. The golden standard for the treatment of LSA is the use of systemic chemotherapy (Vail and Young, 2007; Vail, 2010; Mar-

conato, 2011). Even in cases of stage I nodal or extranodal LSA, systemic spread of the diseases is to be expected within weeks or months after diagnosis (Couto, 2009). In general, dogs treated with chemotherapy are able to enjoy a reasonable quality of life and most of them only experience mild side effects that can usually be treated at home (Mellanby *et al.*, 2003). Combination chemotherapy protocols are superior to single-agent protocols. Most multi-agent chemotherapy protocols are modifications of CHOP protocols used in human medicine for people with high-grade LSA, consisting of cyclophosphamide (C), doxorubicin (hydroxydaunorubicin, H), vincristine (Oncovin, O) and prednisone (P) (Vail and Young, 2007). Many variations of this protocol are available, all of which have very similar disease-free intervals and overall survival times (Vail, 2010). The protocol most commonly used at our Department of Small Animal Medicine and Clinical Biology is the L-VCA-Short protocol. L-asparaginase is not administered in week one, as several studies have proven that adding L-asparaginase does not significantly improve the remission rates and duration. Moreover, it is a drug best reserved for rescue protocols (Vail and Young, 2007). The expected remission rates are 60% to 90% CR, and the expected MST is 275 days for dogs with ML (Hosoya et al., 2007; Couto, 2009; Vail, 2010). However, the remisson rates and MST were lower for six of the dogs of the present study. A possible explanation for five of the cases is that one or more of the following negative prognostic factors were present: stage V, substage b, T-cell type, mediastinal LA or previous treatment with corticosteroids. One of the five dogs additionally suffered from endocarditis and thrombocytopenia, and died eleven days after the diagnosis of LSA was made. In only one of the six cases, none of the negative prognostic factors were present at the time of diagnosis, and the dog survived for another 273 days.

Sometimes, clients lack the financial resources or the time to pursue a multi-agent chemotherapy protocol. In those cases, single-agent therapy is an option. Doxorubicin is the most effective and most commonly used drug for single-agent chemotherapy in dogs with LSA. It is administered intravenously five times with three weeks interval and may induce CR in 50% to 85% of the cases with an MST of seven months (Simon et al., 2008; Chun, 2009; Vail, 2010). One dog with ML of the study was treated with this protocol, and had a survival time of 129 days. However, the dog had previously been treated with a multi-agent chemotherapy protocol by the referring veterinarian. In cases of epitheliotropic LSA, lomustine (CCNU) is frequently used (Risbon et al., 2006; Williams et al., 2006; Vail, 2010).

Side effects in this study related to chemotherapy were present in six dogs (60%). SHC from cyclophosphamide (Chun, 2009) was encountered in one of these dogs. To prevent SHC, furosemide (1 mg/kg IV) is administered simultaneously with cyclophosphamide, and owners are advised to encourage water intake and allow their dogs out to urinate frequently for three days after drug administration. After the development of SHC, the administration of cyclophosphamide is discontinued. In the present study, serum liver enzymes concentrations were increased in one of the dogs treated with lomustine. Hepatopathy is a well-recognized side effect of lomustine treatment (Kristal *et al.*, 2004). In case of grade 2 (1,000-1,499/µl) or grade 4 (<500/µl) neutropenia, the subsequent chemotherapy administration was postponed, and the dose was reduced by 20% in three dogs of the present study. Dose reduction by 20% is only recommended if the neutrophil count is ≤500/µl at its lowest point or <1500/µl at the time the next treatment is scheduled (Vail, 2009). It has been reported that reducing the dose by 20%, the efficacy of the chemotherapy is reduced by 50% (MacDonald, 2009). The dog with grade 4 neutropenia of the present study received enrofloxacin (5 mg/kg/day PO) for seven days. Treatment with a broad-spectrum antibiotic should only be started if the neutrophil count is <1,000/µl, as most companion animals have a low risk of infection as long as their neutrophil count remains greater than 1000/µl (Vail, 2009). Vomiting was treated with maropitant (1 mg/kg SQ once and 2 mg/kg/day PO for two to four days) in three cases, and prophylactic maropitant therapy was given (1 mg/kg SQ once) together with vincristine administrations. In the fourth case of gastrointestinal toxicosis, hospitalisation and fluid therapy were required. This dog suffered from grade 4 gastrointestinal toxicosis and grade 3 thrombocytopenia, but was diagnosed with endocarditis, and euthanized.

In 50% of the cases included in the present study, the owners preferred treatment with corticosteroids only. When excluding the dogs that were euthanized after diagnosis or that had already died before treatment could be initiated, the group treated with prednisolone only accounted for 62% of all the dogs of the study. In these cases, it is important to inform clients that dogs which previously received corticosteroid therapy, are more likely to develop drug-resistant disease if chemotherapy would be considered at a later time (Vail, 2010). It is advised not to start corticosteroid treatment more than 24 to 48 hours prior to starting the chemotherapy. Another disadvantage of the pre-treatment with corticosteroids, is that it may mask the disease and, lead to inaccurate diagnosing or staging at the referral hospital (Ettinger, 2003). Nevertheless, almost 50% of all the dogs of the present study had received corticosteroids prior to being referred, and hence before a definitive diagnosis was established and a therapy was chosen. The MST of the dogs treated with prednisolone in the study (32 days) was comparable to the expected survival time of one to two months in the literature. The MST for the dogs treated with chemotherapy was significantly longer than for the dogs treated with corticosteroids ($P = 0.014$).

In two dogs (3.6%) presented without any clinical signs related to LSA, the histological results revealed the presence of indolent LSA – one splenic and one lingual. Indolent or low-grade LSA is a type of LSA characterized by a low mitotic rate and subsequently a slow clinical progression with a long disease free in-

terval and survival time. Most cases of canine indolent LSA are B-cell tumors, most commonly nodal or splenic. They are not rare, but their incidence is unknown (Valli *et al.*, 2006). A study describing five dogs with splenic marginal zone LSA, showed that dogs undergoing splenectomy and subsequent systemic chemotherapy had long disease free intervals, and eventually died of causes unrelated to lymphoma (Stefanello *et al.*, 2011). Another recent study containing 75 dogs with indolent lymphoma led to the conclusion that systemic treatment does not influence survival, but that further prospective trials are warranted. The overall MST for dogs of that study was 4.4 years (Flood-Kapnik et al., 2012).

CONCLUSION

The results of the present study for signalment, clinical signs and diagnostic techniques correlate well with the previously described results in the literature. At referral hospitals however, the majority of dogs tend to present in late stages and substage b. Treatment with prednisolone was chosen by 50% of the owners or in 62% of the cases where therapy was initiated. The dogs that received chemotherapy had significantly longer survival times than those treated with prednisolone only, although negative prognostic factors were present in the cases treated with chemotherapy.

LITERATURE

Bergmann M., Sauter-Louis C., Hirschberger J. (2011). Dog's quality and prospects of life during chemotherapy for canine lymphoma. An owner survey. *Tierärztliche Praxis Kleintiere 4*, 229-236.

Blackwood L., Sullivan M., Lawson H. (1997). Radiographic abnormalities in canine multicentric lymphoma: a review of 84 cases. *Journal of Small Animal Practice 38*, 62-69.

Blackwood L., German A.J., Stell A.J., O'Neill T. (2004). Multicentric lymphoma in a dog after cyclosporine therapy. *Journal of Small Animal Practice 45*, 259-262.

Brunker J.D., Hoover J.P. (2007). B-cell lymphoma in a dog with ehrlichiosis (Ehrlichia canis) and systemic histoplasmosis (Histoplasma capsulatum). *Canadian Veterinary Journal 48*, 292-295.

Bryan J.N. (2010). Tumors of the hematopoietic system. In: Henry C.J., Higginbotham M.L. (editors). *Cancer Management in Small Animal Practice*. Saunders, Missouri, 343-356.

Couto C.G. (2009). Lymphoma in the cat and dog. In: Nelson R.W., Couto C.G. (editors). *Small Animal Internal Medicine,* 4th ed. Mosby, Edinburgh, 1174-1186.

Cowell R.L., Dorsey K.E., Meinkoth J.H. (2003). Lymph node cytology. *Veterinary Clinics of North America 33*, 47-67.

Chun R. (2009). Lymphoma: which chemotherapy protocol and why*? Topics in Companion Animal Medicine 24*, 157-162.

Durno A.S., Webb J.A., Gauthier M.J., Bienzle D. (2011). Polycythemia and inappropriate erythropoietin concentrations in two dogs with renal T-cell lymphoma. *Journal of the American Animal Hospital Association 47*, 122-128.

Ettinger S.N. (2003). Principles of treatment for canine lymphoma. *Clinical Techniques in Small Animal Practice 18*, 92-97.

Farinha P., Gascoyne R.D. (2005). Helicobacter pylori and MALT lymphoma. *Gastroenterology 128,* 1579-1605.

Flood-Kapnik K.E., Durham A.C., Gregor T.P., Sánchez M.D., Durney M.E., Sorenmo K.U. (2012). Clinical, histopathological and immunohistochemical characterization of canine indolent lymphoma. *Veterinary and Comparative Oncology (Epub ahead of print).*

Flory A.B., Rassnick K.M., Stokol T., Scrivani P.V., Erb H.N. (2007). Stage migration in dogs with lymphoma. *Journal of Veterinary Internal Medicine 21*, 1041-1047.

Garrett L.D., Thamm D.H., Chun R., Dudley R., Vail D.M. (2002). Evaluation of a 6-month chemotherapy protocol with no maintenance therapy for dogs with lymphoma. *Journal of Veterinary Internal Medicine 16*, 704-709.

Gavazza A., Presciuttini S., Barale R., Lubas G., Gugliucci B. (2001). Association between canine malignant lymphoma, living in industrial areas, and use of chemicals by dog owners. *Journal of Veterinary Internal Medicine 15*, 190-195.

Grindem C.B., Neel J.A., Juopperi T.A. (2002). Cytology of bone marrow. *Veterenary Clinics of North America 32*, 1313-1374.

Hawkins E.C., Morrison W.B., DeNicola D.B., Blevins W.E. (1993). Cytologic analysis of bronchoalveolar lavage fluid from 47 dogs with multicentric malignant lymphoma. *Journal of the American Veterinary Medical Association 203*, 1418-1425.

Hosoya K., Kisseberth W.C., Lord L.K., Alvarez F.J., Lara-Garcia A., Kosarek C.E., London C.A., Couto C.G. (2007). Comparison of COAP and UW-19 protocols for dogs with multicentric lymphoma. *Journal of Veterinary Internal Medicine 21*, 1355-1363.

Huang S., Kozak J.P., Kim J., Habineza-Ndikuyeze G., Meade C., Gaurnier-Hausser A., Patel R., Robertson E., Mason N.J. (2012). Evidence of an oncogenic gammaherpesvirus in domestic dogs. *Virology (Epub ahead of print).*

Keller E.T. (1992). Immune-mediated disease as a risk factor for canine lymphoma. *Cancer 70*, 2334-2337.

Keller E.T., MacEwen E.G., Rosenthal R.C., Helfand S.C., Fox L.E. (1993). Evaluation of prognostic factors and sequential combination chemotherapy with doxorubicin for canine lymphoma. *Journal of Veterinary Internal Medicine 7*, 289-295.

Kristal O., Rassnick K.M., Gliatto J.M., Northrup N.C., Chretin J.D., Morrison-Collister K., Cotter S.M., Moore A.S. (2004). Hepatotoxicity associated with CCNU (lomustine) chemotherapy in dogs. *Journal of Veterinary Internal Medicine 18*, 75-80.

MacDonald V. (2009). Chemotherapy: managing side effects and safe handling. *Canadian Veterinary Journal 50*, 665-668.

Marconato L. (2011). The staging and treatment of multicentric high-grade lymphoma in dogs: a review of recent developments and future prospects. *The Veterinary Journal 188(1)*, 34-38.

Marconato L., Stefanello D., Valenti P., Bonfanti U., Comazzi S., Roccabianca P., Caniatti M., Romanelli G., Massari F., Zini E. (2011). Predictors of long-term survival in dogs with high-grade multicentric lymphoma. *Journal of the American Veterinary Medical Association 238(4)*, 480-485.

Mellanby R.J., Herrtage M.E., Dobson J.M. (2003). Owners' assessments of their dog's quality of life during palliative chemotherapy for lymphoma. *Journal of Small Animal Practice 44*, 100-103.

Modiano J.F., Breen M., Burnett R.C., Parker H.G., Inusah S., Thomas R., Avery P.R., Lindblad-Toh K., Ostrander E.A., Cutter G.C., Avery A.C. (2005). Distinct B-cell and T-cell lymphoproliferative disease prevalence among dog breeds indicates heritable risk. *Cancer Research 65*, 5654-5661.

Parodi A., Wyers M., Paris J. (1968). Incidence of canine lymphoid leukosis. Age, sex and breed distribution; results of a necropsic survey. *Bibliotheca Haematologica 30*, 263-267.

Rebhun R.B., Kent M.S., Borrofka S.A.E.B., Frazier S., Skorupski K., Rodriguez C.O. (2010). CHOP chemotherapy for the treatment of canine multicentric T-cell lymphoma. *Veterinary and Comparative Oncology 9,* 38-44.

Risbon R.E., de Lorimier L.P., Skorupski K., Burgess K.E., Bergman P.J., Carreras J., Hahn K., Leblanc A., Turek M., Impellizeri J., Fred R. III, Wojcieszyn J.W., Drobatz K., Clifford C.A. (2006). Response of canine cutaneous epitheliotropic lymphoma to lomustine (CCNU): a retrospective study of 46 cases (1999-2004). *Journal of Veterinary Internal Medicine 20*, 1389-1397.

Santoro D., Marsella R., Hernandez J. (2007). Investigation on the association between atopic dermatitis and the development of mycosis fungoides in dogs: a retrospective case-control study. *Veterinary Dermatology 18*, 101-106.

Simon D., Moreno S.N., Hirschberger J., Moritz A., Kohn B., Neumann S., Jurina K., Scharvogel S., Schwedes C., Reinacher M., Beyerbach M., Nolte I. (2008). Efficacy of a continuous, multiagent chemotherapeutic protocol versus a short-term single-agent protocol in dogs with lymphoma. *Journal of the American Veterinary Medical Association 232*, 879-885.

Starrak G.S., Berry C.R., Page R.L., Johnson J.L., Thrall D.E. (1997). Correlation between thoracic radiographic changes and remission/survival duration in 270 dogs with lymphosarcoma. *Veterinary Radiology and Ultrasound 38*, 411-418.

Stefanello D., Valenti P., Zini E., Comazzi S., Gelain M.E., Roccabianca P., Avallone G., Caniatti M., Marconato L. (2011). Splenic marginal zone lymphoma in 5 dogs (2001-2008). *Journal of Veterinary Internal Medicine 25(1)*, 90-93.

Vail D.M., Kisseberth W.C., Obradovich J.E., Moore F.M., London C.A., MacEwen E.G., Ritter M.A. (1996). Assessment of potential doubling time (Tpot), argyrophilic nucleolar organizer regions (AgNOR), and proliferating cell nuclear antigen (PCNA) as predictors of therapy response in canine non-Hodgkin's lymphoma. *Experimental Hematology 24*, 807-815.

Vail D.M., Young K.M. (2007). Hematopoietic tumors. In: Withrow S.J., Vail D.M. (editors). *Withrow & MacEwen's Small Animal Clinical Oncology,* 4th ed. Saunders, St. Louis, 699-733.

Vail D.M. (2009). Supporting the veterinary cancer patient on chemotherapy: neutropenia and gastrointestinal toxicity. *Topics in Companion Animal Medicine 24*, 122-129.

Vail D.M. (2010). Hematopoietic tumors. In: Ettinger S.J., Feldman E.C. (editors). *Textbook of Veterinary Internal Medicine: Diseases of the Cat and Dog,* 7th ed. Saunders, St. Louis, 2148-2158.

Valli V.E., Vernau W., de Lorimier L.P., Graham P.S., Moore P.F. (2006). Canine indolent nodular lymphoma. *Veterinary Pathology 43*, 241-256.

Veterinary Co-operative Oncology Group (2004). Common terminology criteria for adverse events (VCOG-CTCAE) following chemotherapy or biological antineoplastic therapy in dogs and cats v1.1. *Veterinary and Comparative Oncology 2(4)*, 195-213.

Webb J.L., Rakich P.M., Latimer K.S. (2008). The gastrointestinal tract. In: Cowell R.L., Tyler R.D., Meinkoth J.H., DeNicola D.B. (editors). *Diagnostic Cytology and Hematology of the Dog and Cat,* 3rd ed. Mosby, St. Louis, 288-299.

Williams L.E., Rassnick K.M., Power H.T., Lana S.E., Morrison-Collister K.E., Hansen K., Johnson J.L. (2006). CCNU in the treatment of canine epitheliotropic lymphoma. *Journal of Veterinary Internal Medicine 20*, 136-143.