Luteinizing hormone receptor expression in lymphoma is not affected by body weight, sex, immunophenotype, or tumor stage in gonadectomized dogs

Khawla Zwida, Alyssa Vedus, Michelle Kutzler
Department of Animal and Rangeland Sciences, Oregon State University, Corvallis, OR

Abstract

Purpose of this investigation was to determine if luteinizing hormone receptor (LHR) expression in canine lymphoma was affected by body weight, sex, immunophenotype (B cell, T cell) or tumor stage. Formalin-fixed, paraffin-embedded lymphoma tissue samples from spayed and neutered dogs (n = 40), representing multiple breeds and mixed breeds, were subjected to routine immunohistochemical techniques using a polyclonal LHR antibody. Percentage of cells positive for LHR and the staining intensity (scored 0 - 3) were determined at 400 x magnification. Data were expressed as mean ± standard deviation and significance was defined as $p < 0.05$. Differences between sex and tumor phenotype, body weight and tumor stage were compared. All tumor samples had cells positive for LHR. However, percentage of cells expressing LHR and its immunostaining intensity varied among individuals. There were no significant differences in percentage of LHR positive cells or staining intensity within sex or immunophenotype. Additionally, there was no significant association between percentage of LHR positive cells or staining intensity within body weight or tumor stage. We concluded that increased risk of lymphoma in spayed and neutered dogs was not related to body weight, sex, immunophenotype or tumor stage. However, it is possible that risk of lymphoma may be related to increased LHR activation following gonadectomy.

Keywords: B cell, T cell**,** gonadectomized dogs, lymphoma, immunohistochemistry

Introduction

Canine lymphoma is the most common hematopoietic neoplasm in the dog, accounting for up to 24% of all canine cancers.[1,2] Lymphoma can be derived from either B cell or T cell lymphocytes. Immunophenotyping is possible because B cell lymphomas typically express cell surface proteins CD79a, CD20, CD21, and PAX5, whereas T cell lymphomas typically express the unique cell surface protein CD3.[3] Symptoms and treatment options vary depending on the lymphoma's predominant cell type (i.e. B or T cells).[4] Although canine lymphoma is typically treated by chemotherapy, B cell lymphomas become resistant to chemotherapy after subsequent treatments and T cell lymphomas often do not respond well to chemotherapy from the onset of treatment.[5] Prognosis following diagnosis with canine lymphoma is also dependent on the immunophenotype of lymphoma, with average survival times shorter for T cell than B cell lymphomas (183 versus 365 days).[6] In general, T cell lymphomas also have shorter remissions than B cell lymphomas.[7-9]

Basset Hound, Beagle, Bernese Mountain Dog, Boxer, Bulldog, Bull Mastiff, Cocker Spaniel, Doberman, German Shepherd, Golden Retriever, Labrador Retriever, Rottweiler, Shih Tzu, St. Bernard, and any terrier breed are at higher risk for developing lymphoma, whereas Dachshunds and Pomeranians are relatively lower risk breeds.[2,8,10,11] Additionally, breed appears to influence the lymphoma immunophenotype. Boxers are more likely to develop T cell lymphomas, whereas Basset Hounds and Cocker Spaniels are more likely to develop B cell lymphomas.[12] Body weight may or may not be a risk factor for lymphoma. Although body weight in mixed breed dogs had no influence in lymphoma occurrence,[13] it had an influence in purebred dogs; [9] lymphomas were more common in medium and large breed dogs compared to toy and small breed dogs.[9] Additionally, smaller body weight dogs (< 17 kg) were reported to have longer median survival times[14,15] or no difference in median survival times[1,16] when compared to dogs with greater body weight. However, body condition was not reported in these studies. It is noteworthy that dogs with an underweight body condition had significantly shorter survival times than dogs in ideal or overweight body condition.[17]

Survival from lymphoma may be influenced by sex. In humans, men generally had shorter survival times than women for most cancers, including lymphoma.[18-20] This is similar to what was

observed in dogs with advanced multicentric lymphoma; intact male dogs had significantly shorter remission and survival times than intact female dogs.[10]

Luteinizing hormone receptors (LHR) are expressed in canine lymphoma tissue and isolated canine lymphoma cells.[21] However, the influence of body weight or sex on LHR expression was not evaluated. Additionally, the influence of immunophenotype or tumor stage on LHR expression was also not evaluated. Based on canine lymphoma findings,[6-10,14,15] it was hypothesized that greater body weight male dogs in advanced tumor stages with T cell lymphomas have higher LHR expression than lesser body weight female dogs in earlier tumor stages with B cell lymphomas.

Materials and methods

Archived tissue samples (Canine Comparative Oncology and Genomics Consortium [Bethesda, MD]) from 40 purebred and mixed breed dogs (spayed females n = 24; neutered males n = 16) weighing 7 - 56 kg were used. Weight categories were similar to an earlier study;[13] < 10 kg (n = 4), 10 - 19 kg (n = 8), 20 - 29 kg (n =14), 30 - 39 kg (n = 10), and > 40 kg (n = 4). Immunophenotype was available for 26 of 40 tumors (Table). Tumors were staged using the World Health Organization system: Stage I, single node or lymphoid tissue in single organ; Stage II, regional multiple lymph nodes involvement; Stage III, generalized lymph node involvement; Stage IV, Stages I - III with liver and/or spleen involvement; and Stage V, Stages I - IV with blood or bone marrow involvement.[21] Additionally, samples from each primary tumor were formalin-fixed, paraffin-embedded, and sectioned onto charged slides for LHR immunohistochemistry. All slides were deparaffinized, rehydrated, subjected to heat-induced epitope retrieval (#S1700, Dako, Carpinteria, CA). Endogenous peroxidase activity was inactivated with 3% hydrogen peroxide and nonspecific binding was blocked (Protein Block Serum-Free [#X0909, Dako]). Either rabbit polyclonal antihuman LHR antibody (#NLS1436, Novus Biologics, Centennial, CO) was applied at 1:100 dilution or rabbit negative control (#NC495H, Biocare Medical, Pacheco, CA) was applied to slides. This antigen retrieval method and antibody concentration were optimal for normal and neoplastic canine lymphatic tissue.[21] Slides were treated with One Step Horse Radish Peroxidase-Conjugated Polymer Antirabbit IgG (#IH-8064-custom-OrSU, ImmunoBioScience, Mukilteo, WA), followed by Nova Red Peroxidase substrate (#SK4800, Vector Laboratories, Burlingame, CA). Slides were counter-stained with hematoxylin, dehydrated, and mounted. Percentage of cells positive for LHR was recorded and staining intensity (scored 0 - 3; Figure 1) was determined at 400 x magnification from 5 randomly selected fields by 1 person (AV) blinded to the identity of the samples. Additionally, a histology score (h-score) was assigned to each tumor sample.[23,24] Staining intensity (0 - 3) for a fixed field was multiplied by the percentage of cell positive for LHR.

Differences between sex and tumor phenotype were compared (Welch two sample t-test in the free statistical package R [Version 1.2.1355, Boston, MA]). Differences in bodyweight and tumor stage were compared (simple linear regression [Microsoft Excel, Version 14.5.2, Redmond, WA]). Additionally, differences in body weight group were compared (one-way ANOVA). Data were expressed as mean ± standard deviation and significance was defined as $p < 0.05$.

Results

Although there were many purebred dogs included in the data set, mixed breed dogs were overrepresented (Table). Most breeds were only represented by 1 individual, except Labrador retrievers (n = 3), German shorthaired pointers (n = 2), poodles (n = 2), miniature schnauzers (n = 2), and Boston terriers (n = 2). Irrespective of breed, all lymphoma tissue samples contained cells positive for LHR. However, percentage of cellular expression and staining intensity varied among individuals (Figure 1). Body weight had no significant influence and there was no significant association between the percentage of LHR positive cells ($R^2 = 0.021$) or staining intensity ($R^2 = 0.077$). There were also no significant differences in the percentage of LHR positive cells, staining intensity or h-score when compared by sex (Figure 2) or lymphoma phenotype (Figure 3). There was no significant association between the percentage of LHR positive cells ($R^2 = 0.0017$) or staining intensity ($R^2 = 0.063$) with regard to tumor stage.

Table. Gonadectomized dogs with lymphoma studied: body weight, sex, tumor phenotype, tumor stage on the percentage of LHR positive cells, staining intensity, and histology score (h-score) Female (F); Male (M); Not available (NA)

ID	Breed	Sex	Weight (kg)	Tumor phenotype	Tumor stage	Percent positive	Staining intensity	h-score
5678	Miniature Schnauzer	F	7	NA	3	10	2.5	25
5682	Shih Tzu	F	7.2	NA	5	10.5	1	10.5
5656	Boston Terrier	F	8	NA	2	10.5	3	31.5
5664	Pembroke Welsh Corgi	F	9.5	NA	3	6.8	3	20.4
5676	Miniature Schnauzer	F	11.4	NA	3	3	2.5	7.5
5698	Shetland Sheepdog	F	12.6	B cell	3	23.5	3	70.5
5707	Cocker Spaniel	F	14	T cell	3	10.3	3	30.7
5657	Poodle	M	15.2	B cell	5	5.75	2.5	14.4
5702	Boston Terrier	M	16	B cell	3	20	2	40
5675	Mixed breed	F	17.3	NA	5	16.6	2	33.2
5666	Vizsla	F	19	B cell	3	20.8	1.5	31.2
5713	Mixed breed	F	19.7	NA	3	7.5	3	22.5
5689	Labrador Retriever	F	21	T cell	5	7.5	2	15
5715	Mixed breed	M	21.4	B cell	3	3	2	6
5709	Poodle	F	22	B cell	4	3.6	2	7.2
5708	Mixed breed	F	22.6	B cell	3	9	2.5	22.5
5662	German Shorthair Pointer	F	24.5	NA	4	10.8	3	32.4
5672	German Shorthair Pointer	F	26	NA	4	9.5	2	19
5681	Mixed breed	M	26	B cell	4	21.8	2	43.6
5655	Samoyed	F	26.7	NA	2	17.65	3	52.9
5691	Boxer	M	28.6	T cell	3	15	2	30
5659	Labrador Retriever	F	28.6	NA	2	19	2	38
5710	Mixed breed	F	28.8	B cell	3	6.5	2	13
5684	Dalmatian	M	29	B cell	3	9	2	18
5714	Mixed breed	M	29.3	B cell	4	2	1.5	3
5720	Mixed breed	M	29.5	B cell	4	10	1.5	15
5694	Bernese Mountain Dog	M	30	T cell	3	4.3	1.5	6.4
5660	Labrador Retriever	F	30	T cell	5	5.9	2	11.8
5668	Mixed breed	M	31.6	B cell	4	13.4	2	26.8
5690	Mixed breed	M	32	B cell	3	7.8	2	15.6
5667	Basset Hound	F	34.6	NA	3	8.2	3	24.6
5658	Australian Shepherd	M	35.2	T cell	5	10.3	2	20.6
5688	Mixed Breed	M	35.2	B cell	3	10	1	10
5671	Mixed breed	F	37.2	B cell	4	5	3	15
5706	Black/Tan Coonhound	F	37.5	NA	3	3.6	2	7.2
5704	Mixed breed	F	38	B cell	4	26.5	0.5	13.2
5685	Irish Setter	M	46.5	B cell	4	7	3	21
5703	Mixed breed	M	50	B cell	3	8	2	16
5680	Bullmastiff	F	52	B cell	5	7.3	0.5	3.6
5705	Saint Bernard	M	56	NA	5	6.3	2.5	15.6

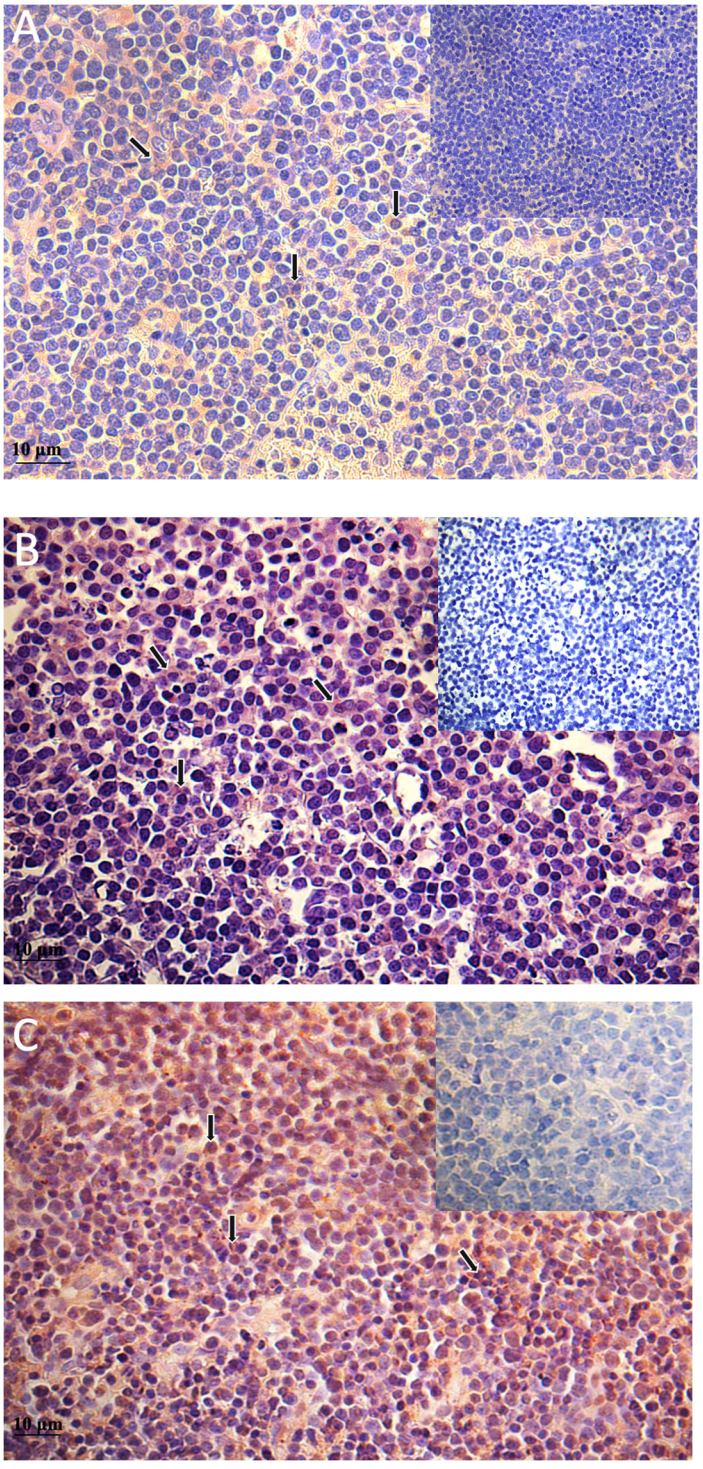

Figure 1. Representative images for luteinizing hormone receptors staining intensity in canine lymphoma (A: staining intensity = 1; B: staining intensity = 2; C: staining intensity = 3). Nova red; Bar = 10 µm. Negative control in upper right inset. Arrows illustrate examples of cells stained positive.

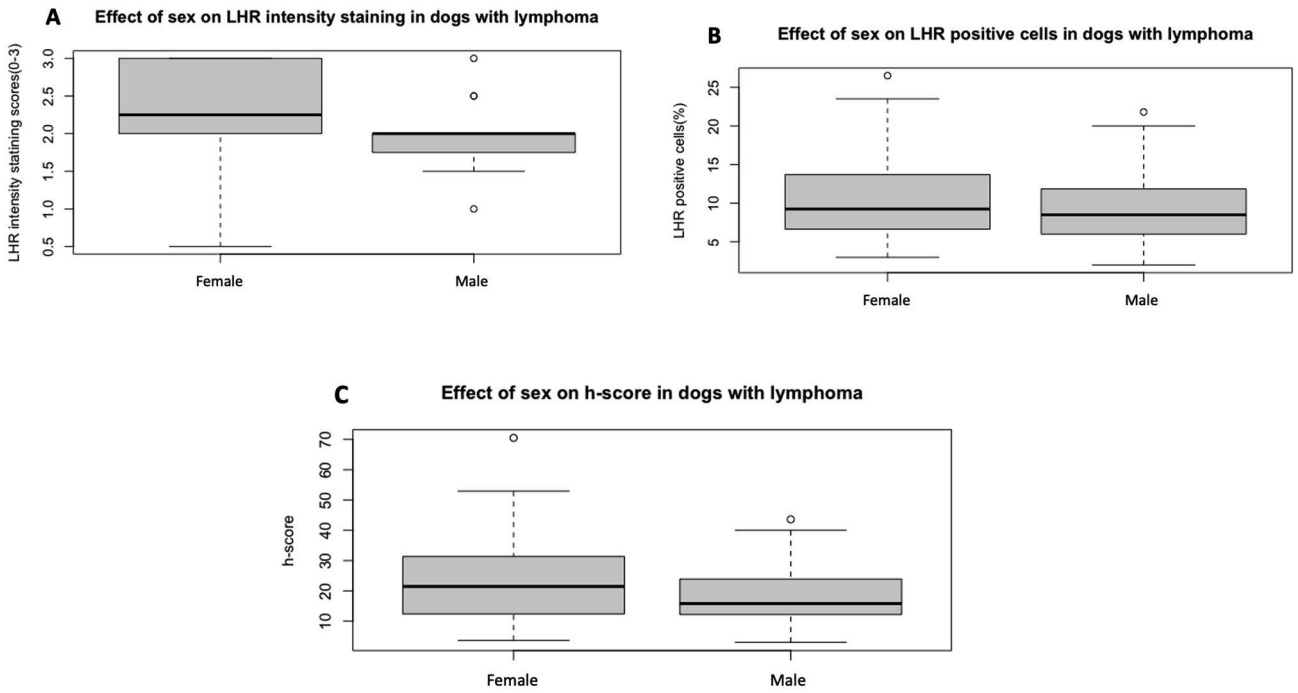

Figure 2. Effect of sex (neutered male versus spayed female) on luteinizing hormone receptor (LHR) staining intensity (A), percentage of positive cells (B), and h-score (C). There was no significant effect of sex on LHR expression ($p > 0.05$).

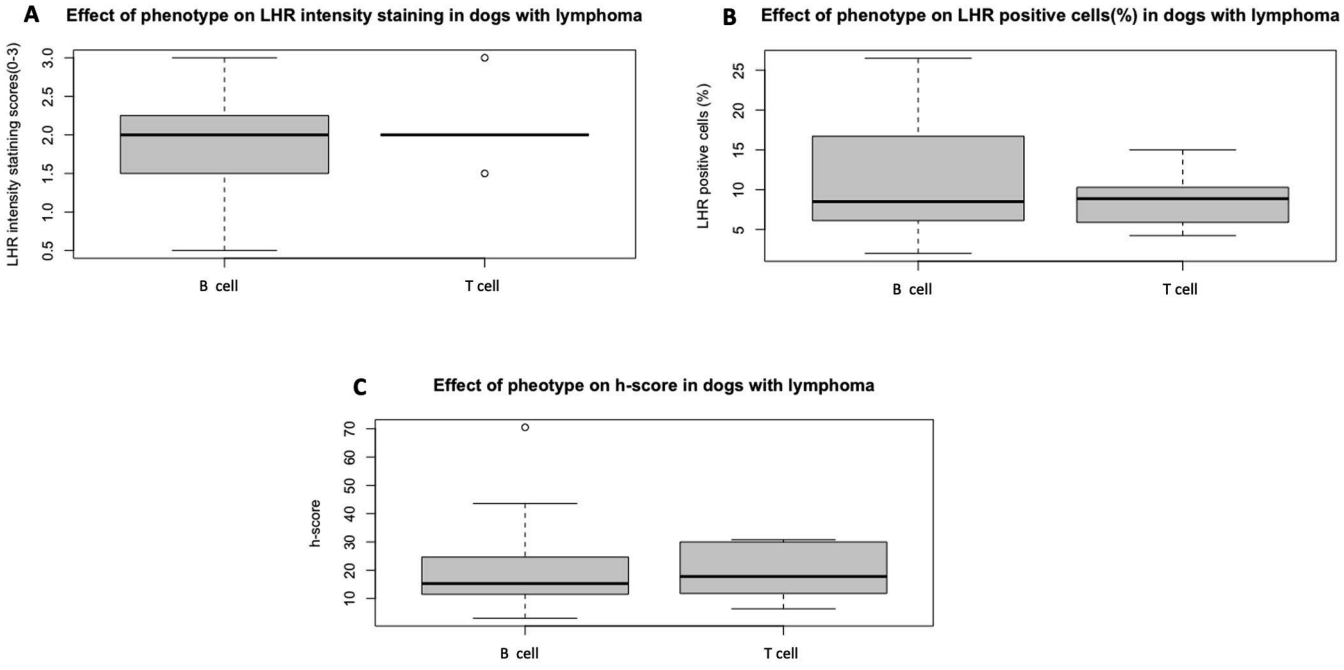

Figure 3. Effect of lymphoma phenotype (B cell versus T cell) on luteinizing hormone receptor (LHR) staining intensity (A) and percentage of positive cells (B), and h-score (C). There was no significant effect of phenotype on LHR expression ($p > 0.05$).

Discussion

Certain breeds are predisposed to developing lymphoma.[2,8-12,25] Since there were not enough dogs represented by any single breed in the current study to compare the expression of LHR, the effect of body weight was examined. In agreement with previous research on LHR expression in the bladder,[26] body weight did not influence LHR expression in canine lymphoma. It is important to note that body condition score was not available in the current study, which could have been a confounding factor, since underweight dogs with lymphoma were reported to have significantly shorter survival times.[17]

Sex had a role in remission and survival times following lymphoma diagnosis.[1,9,16,27] Therefore, we sought to determine if LHR expression in canine lymphoma differed by sex. Although there was no significant influence of sex on LHR expression, it is noteworthy that the case material available for this study was unfortunately limited to only spayed and neutered dogs; these dogs may have an increased risk of developing lymphoma, depending on their breed.[25] Compared to intact females and males, lymphoma is 4 times more common in spayed and neutered Viszlas.[28] Occurrence of lymphoma was higher in spayed than intact females. [10] Absence of gonadal hormones in the dogs sampled for the current study may have masked effects of sex on LHR expression. Further research is needed on susceptible breeds to determine if LHR expression within lymphoma tissue is increased in gonadectomized compared to intact dogs.

Lymphoma phenotype often influences receptor expression. For example, most human B cell lymphomas depended on the expression of a B cell receptor for continued growth.[29] In dogs, the retinoid receptor is expressed higher in T cell lymphomas compared to B cell lymphomas.[30] Therefore, 1 objective of the current study was to determine if LHR expression changed with lymphoma phenotype. Although more B cell lymphomas were present in the data set (n = 20) than T cell lymphomas (n = 6), there was no significant difference between phenotypes in the relative percentage of cells expressing LHR, nor in the relative intensity of LHR staining.

Receptor expression also can vary with advancing tumor stage. For example, estrogen receptor beta was expressed higher in human B cell lymphoma with tumor stage of III or IV.[31] Alternatively, LHR expression in human ovarian cancer was higher in Stages I and II compared to Stages III and IV.[32] In the current study, LHR expression varied widely within tumor stage, similar to reported expression of steroid hormone receptors in canine and human mammary tumors.[33-35] Lack of association between receptor expression and tumor stage could result from the nonstatic expression of LHR in tumors, as reported with lipoprotein receptors in canine lymphoma.[36]

Activation of LHR in luteal,[37,38] trophoblast,[39] or canine T cell lymphoma cells[40] resulted in dose-dependent cell proliferation. Because circulating LH concentrations were significantly higher in spayed and neutered dogs,[41] it is possible that LHR activation has a role in the etiopathogenesis of canine lymphoma. Additional research is needed to determine if reducing LH concentrations in spayed and neutered dogs with a gonadotropin-releasing hormone agonist prolongs survival time in dogs with canine lymphoma.

Acknowledgement

Pfizer Canine Comparative Oncology and Genomics Consortium Biospecimen Repository for access to study specimens and Christina Mazcko for assistance with patient data.

Conflict of interest

Authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

Oregon State University small grants incentive program and undergraduate research scholarship and arts program.

References

1. MacEwen EG, Hayes AA, Matus RE, et al: Evaluation of some prognostic factors for advanced multicentric lymphosarcoma in the dog: 147 cases (1978-1981). J Am Vet Med Assoc 1987;190:564-568.
2. Vail DM, MacEwen, EG, Young KM: Canine lymphoma and lymphoid leukemia. In: Withrow SJ, MacEwen EG: editors. Small Animal Clinical Oncology, 3nd edition, Philadelphia; WB Saunders: 2001. p. 558-590.
3. Caniatti M, Roccabianca P, Scanziani E, et al: Canine lymphoma: immunocytochemical analysis of fine-needle aspiration biopsy. Vet Pathol 1996;33:204-212.
4. Burke, A: Lymphoma in dogs: symptoms, diagnosis, and treatment. American Kennel Club. November 3, 2016. Accessed April 22, 2018. Available from: https://www.akc.org/expert-advice/health/common-conditions/lymphoma-in-dogs-symptoms-diagnosis-and-treatment/.
5. Marconato L, Gelain ME, Comazzi S: The dog as a possible animal model for human non-Hodgkin lymphoma: a review. Hematol Oncol 2013;31:1-9.
6. Fan TM: Overview of canine lymphoma - circulatory system. MVM. 2018. Accessed April 21, 2018. Available from: https://www.merckvetmanual.com/circulatory-system/canine-lymphoma/overview-of-canine-lymphoma.
7. Greenlee PG, Filippa DA, Quimby FW, et al: Lymphomas in dogs. a morphologic, immunologic, and clinical study. Cancer 1990;66:480-490.
8. Teske E. Canine malignant lymphoma: a review and comparison with human non-Hodgkins lymphoma. Vet Q 1994;16:209-219.
9. Valli VE, Kass PH, San Myint M, et al: Canine lymphomas. Vet Path 2013; 50;738-748.
10. Villamil JA, Henry CJ, Hahn AW, et al: Hormonal and sex impact on the epidemiology of canine lymphoma. J Cancer Epidemiol 2009;2009:591753.
11. Comazzi S, Marelli S, Cozzi M, et al: Breed-associated risks for developing canine lymphoma differ among countries: an European canine lymphoma network study. BMC Vet Res 2018;14:232.
12. Dobson, JM: Breed-predispositions to cancer in pedigree dogs. ISRN Vet Sci 2013;2013:941275.
13. Hart BL, Hart LA, Thigpen AP, et al: Assisting decision-making on age of neutering for mixed breed dogs of five weight categories: associated joint disorders and cancers. Front Vet Sci 2020;7:472.
14. Garrett LD, Thamm DH, Chun R, et al: Evaluation of a 6-month chemotherapy protocol with no maintenance therapy for dogs with lymphoma. J Vet Intern Med 2002;16:704-709.
15. Valerius KD, Ogilvie GK, Mallinckrodt CH, et al: Doxorubicin alone or in combination with asparaginase, followed by cyclophosphamide, vincristine, and prednisone for treatment of multicentric lymphoma in dogs: 121 cases (1987-1995). J Am Vet Med Assoc 1997;210:512-516.
16. Deravi N, Berke O, Woods JP, et al: Specific immunotypes of canine T cell lymphoma are associated with different outcomes. Vet Immunol Immunopathol 2017;191:5-13.
17. Romano FR, Heinze CR, Barber LG, et al: Association between body condition score and cancer prognosis in dogs with lymphoma and osteosarcoma. J Vet Intern Med 2016;30:1179-1186.
18. Afshar N, English DR, Thursfield V, et al: Differences in cancer survival by sex: a population-based study using cancer registry data. Cancer Causes Control 2018;29:1059-1069.
19. Radkiewicz C, Johansson ALV, Dickman PW, et al: Sex differences in cancer risk and survival: A Swedish cohort study. Eur J Cancer 2017;84:130-140.
20. Cook MB, Katherine AM, Susan SD, et al: "Sex disparities in cancer mortality and survival." Cancer Epidemiol Biomarkers Prev 2011;20:1629-1637.
21. Ettinger AM, Gust SK, Kutzler MA: Luteinizing hormone receptor expression by nonneoplastic and neoplastic canine lymphocytes. Am J Vet Res 2019;80:572-577.
22. Owen LN: TNM classification of tumours in domestic animals. 1st edition, Geneva; World Health Organization: 1980.
23. Hirsch FR, Varella-Garcia M, Bunn PA Jr, et al: Epidermal growth factor receptor in non-small-cell lung carcinomas: Correlation between gene copy number and protein expression and impact on prognosis. J Clin Oncol 2003;21: 3798-3807.
24. John T, Liu G, Tsao MS: Overview of molecular testing in non-small-cell lung cancer: Mutational analysis, gene copy number, protein expression and other biomarkers of EGFR for the prediction of response to tyrosine kinase inhibitors. Oncogene 2009;28:S14-S23.
25. Hart BL, Hart LA, Thigpen AP, et al: Assisting decision-making on age of neutering for 35 breeds of dogs: associated joint disorders, cancers, and urinary incontinence. Front Vet Sci 2020;7:388.
26. Reichler IM, Welle M, Sattler U, et al: Comparative quantitative assessment of GnRH- and LH-receptor mRNA expression in the urinary tract of sexually intact and spayed female dogs. Theriogenology 2007;67:1134-1142.
27. Keller ET, MacEwen EG, Rosenthal RC, et al: Evaluation of prognostic factors and sequential combination chemotherapy with doxorubicin for canine lymphoma. J Vet Intern Med 1993;7:289-295.
28. Zink MC, Farhoody P, Elser SE, et al: Evaluation of the risk and age of onset of cancer and behavioral disorders in gonadectomized Vizslas. J Am Vet Med Assoc 2014;244:309-319.
29. Küppers R: Mechanisms of B-cell lymphoma pathogenesis. Nat Rev Cancer 2005;5:251-262.
30. de Mello SCH, Valli VE, Kitchell BE: Detection of retinoid receptors in non-neoplastic canine lymph nodes and in lymphoma. Can Vet J 2014;55:1219-24. Erratum in: Can Vet J 2014;55:106.

31. Faknuam S, Assanasen T, Ruangvejvorachai P, et al: Estrogen receptor beta expression and prognosis of diffuse large B cell lymphoma. Hematology 2018;23:235-241.
32. Cui J, Guo Y, You Z: [LH-CG receptor protein expression in epithelial ovarian cancer]. Zhonghua Fu Chan Ke Za Zhi 1997;32:742-745.
33. Millanta F, Calandrella M, Bari G, et al: Comparison of steroid receptor expression in normal, dysplastic, and neoplastic canine and feline mammary tissues. Res Vet Sci 2005;79:225-232.
34. Bardou VJ, Arpino G, Elledge RM, et al: Progesterone receptor status significantly improves outcome prediction over estrogen receptor status alone for adjuvant endocrine therapy in two large breast cancer databases. J Clin Oncol 2003;21:1973-1979.
35. Pichon MF, Broet P, Magdelenat H, et al: Prognostic value of steroid receptors after long-term follow-up of 2257 operable breast cancers. Br J Cancer 1996;73:1545-1551.
36. Ceres K, Fitzgerald H, Quiznon KS, et al: Immunohistochemical labeling of low-density lipoprotein receptor and scavenger receptor class b type 1 are increased in canine lymphoma. Front Vet Sci 2019;11:340.
37. Menon K: Regulation of luteinizing hormone receptor expression by an RNA binding protein: Role of ERK signaling. Indian J Med Res 2014;140, Suppl S1:112-119.
38. Davis JS, May JV, Keel BA: Mechanisms of hormone and growth factor action in the bovine corpus luteum. Theriogenology 1996;45:1351-1380.
39. Reshef E, Lei ZM, Rao CV, et al: The presence of gonadotropin receptors in nonpregnant human uterus, human placenta, fetal membranes, and decidua. J Clin Endocrinol Metab 1990;70:421-430.
40. Flint C, Gust S, Kutzler MA: Luteinizing hormone receptor mediated proliferation of isolated canine T lymphoma cells. Clinical Theriogenology 2019;11:477.
41. Beijerink NJ, Buijtels JJ, Okkens AC, et al: Basal and GnRH-induced secretion of FSH and LH in anestrous versus ovariectomized bitches. Theriogenology 2007;67:1039-1045.