



Serum vascular endothelial growth factor in dogs with various proliferative diseases

Hiro HORIKIRIZONO^{1,2}), Kumiko ISHIGAKI¹), Keigo IIZUKA¹), Kei TAMURA¹), Naoki SAKURAI¹), Kazuyuki TERAJ¹), Tatsuya HEISHIMA¹), Orié YOSHIDA¹) and Kazushi ASANO¹)*

¹)Laboratory of Veterinary Surgery, Department of Veterinary Medicine, College of Bioresource Sciences, Nihon University, Kanagawa, Japan

²)Present address: Department of Veterinary Radiology, Joint Faculty of Veterinary Medicine, Yamaguchi University, Yamaguchi, Japan

ABSTRACT. Angiogenesis plays an important role in the proliferation and metastasis mechanisms of malignant tumors. Vascular endothelial growth factor (VEGF), a group of cytokines that contribute to angiogenesis and vasculogenesis. This study aimed to investigate the serum VEGF-A concentrations in dogs with various proliferative diseases. A total of 202 dogs that were histopathologically diagnosed with proliferative diseases were included in the study. Serum VEGF-A concentrations were measured using enzyme-linked immunosorbent assay. Median serum VEGF-A concentrations in dogs were as follows: healthy dogs, 4 pg/ml [0–21 pg/ml]; hepatocellular carcinoma, 30 pg/ml [0–158 pg/ml, $P < 0.001$]; hepatocellular adenoma, 32 pg/ml [0–49 pg/ml, $P = 0.003$]; hepatic nodular hyperplasia, 18 pg/ml [0–51 pg/ml, $P = 0.595$]; adrenal pheochromocytoma, 32 pg/ml [0–187 pg/ml, $P < 0.001$]; adrenocortical carcinoma, 32 pg/ml [3–161 pg/ml, $P = 0.002$]; adrenocortical adenoma, 27 pg/ml [0–106 pg/ml, $P = 0.005$]; colorectal adenocarcinoma, 36 pg/ml [0–75 pg/ml, $P = 0.002$]; colorectal adenoma, 43 pg/ml [0–48 pg/ml, $P = 0.144$]; inflammatory colorectal polyps, 37 pg/ml [0–111 pg/ml, $P < 0.001$]; pulmonary adenocarcinoma, 35 pg/ml [4–107 pg/ml, $P = 0.002$]; pulmonary histiocytic sarcoma, 35 pg/ml [0–131 pg/ml, $P = 0.016$]; and follicular thyroid carcinoma, 35 pg/ml [0–106 pg/ml, $P = 0.009$]. The serum VEGF-A concentrations were significantly higher in dogs with neoplastic lesions compared to healthy dogs, except for colorectal adenoma. High serum VEGF-A concentrations were observed in dogs with proliferative diseases. The present study suggests that angiogenesis-inhibiting therapy, which targets VEGF-A, may be useful for canine neoplastic diseases.

KEYWORDS: angiogenesis, dog, molecular-targeted therapy, tumor, vascular endothelial growth factor

J. Vet. Med. Sci.
84(5): 720–725, 2022
doi: 10.1292/jvms.21-0509

Received: 21 September 2021
Accepted: 17 March 2022
Advanced Epub:
4 April 2022

Angiogenesis plays an important role in the proliferation and metastasis mechanisms of malignant tumors. The growth of solid carcinoma is always accompanied by angiogenesis in the vicinity of the tumor [10]. Metastatic cancer cells receive signals to proliferate, create new blood vessels (angiogenesis), and then successfully grow into a measurable metastatic lesion [2]. Vascular endothelial growth factor (VEGF), a group of cytokines that contribute to angiogenesis and vasculogenesis, includes VEGF-A, VEGF-B, VEGF-C, VEGF-D, and VEGF-E. Of the VEGF family, VEGF-A promotes angiogenesis by binding to VEGF receptors (VEGFR)-1 and VEGFR-2, which are both expressed in vascular endothelial cells and are thought to be the most important factor in tumor proliferation and metastasis [28].

Previous studies have shown that blood VEGF-A concentration is higher in humans with colorectal cancer [18, 20, 41], hepatocellular carcinoma [16, 21, 27], breast cancer [1], and prostate cancer [39] than in healthy individuals. Additionally, it has been demonstrated that blood VEGF-A concentration is correlated with prognosis and stage in patients with colorectal cancer [4, 18, 20, 41], hepatocellular carcinoma [16, 21, 29, 42], non-small cell lung cancer [9], breast cancer [1, 24], and non-Hodgkin's lymphoma [35]. Furthermore, molecular-targeted therapy, which uses antibodies to inhibit the angiogenesis and proliferation of tumor tissues by specifically binding to VEGF-A and selectively inhibiting VEGFR-1 and VEGFR-2 binding, has been used for colorectal cancer [7, 15, 34] and non-small cell lung cancer [36].

Canine VEGF and VEGFR have similar amino acid sequences to those in humans, and it has also been shown that the VEGF signal transduction pathways in dogs and humans are remarkably similar [38]. Additionally, a correlation between VEGF-A and

*Correspondence to: Asano, K.: asano.kazushi@nihon-u.ac.jp, Laboratory of Veterinary Surgery, Department of Veterinary Medicine, College of Bioresource Sciences, Nihon University, 1866 Kameino, Fujisawa, Kanagawa 252-0880, Japan
(Supplementary material: refer to PMC <https://www.ncbi.nlm.nih.gov/pmc/journals/2350/>)

©2022 The Japanese Society of Veterinary Science



This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial No Derivatives (by-nc-nd) License. (CC-BY-NC-ND 4.0: <https://creativecommons.org/licenses/by-nc-nd/4.0/>)

VEGFR expression in canine tumor tissues and microvessel density has been reported in mammary tumors [30, 32], seminomas [31], and squamous cell carcinomas [23], and high ascitic and urinary VEGF concentrations have been observed in canine malignant tumors [5, 26]. Serum VEGF-A concentrations have been reported to be higher in dogs with mammary tumors [17], angiosarcomas [6, 12], and lymphomas [13], relative to those in healthy dogs. Furthermore, serum VEGF-A concentrations are known to decrease following surgical resection in cases of soft tissue sarcoma [8]. Although VEGF-A-targeting treatment shows anti-tumor effects in mice xenografted with canine osteosarcoma cells and hemangiopericytomas [25, 37], its effectiveness is still unclear for canine proliferative diseases in clinical settings. The proliferative disease with high serum VEGF-A concentration may be a candidate for VEGF-A-targeting treatment. Thus, the present study aimed to investigate serum VEGF-A concentrations in dogs with proliferative diseases and conduct a comparative analysis.

MATERIALS AND METHODS

Animals

A total of 202 dogs undergoing surgical treatment for diseases relating to hepatic, adrenal, colorectal, pulmonary, and thyroid tumors in Animal Medical Center, Nihon University from February 2012 to October 2018 were included in this study. Histopathological diagnoses were reviewed by one veterinary pathologist (YK). Dogs with other systemic diseases or multiple type of proliferative diseases were excluded from this study. Additionally, 13 healthy adult beagles without any specific diseases suspected on physical examination, complete blood count, blood chemistry, and radiography, were used as healthy group. These dogs were managed in accordance with the Nihon University College of Bioresource Sciences animal experiment guidelines and procedures, and the present study was conducted with the approval of the Nihon University animal experiment ethics committee (number AP18BRS041-1).

Blood serum collection methods and processing

Blood sampling of dogs with proliferative diseases was conducted from the initial diagnosis to surgery. Venous blood collection was conducted either through the jugular vein or lateral saphenous vein in both dogs with proliferative diseases and those in the healthy group. The collected blood samples were left to stand for 30 min in vacuum-sealed blood collection tubes (Venoject®II vacuum-sealed blood collection tubes, Terumo, Tokyo, Japan). The samples were then centrifugally separated at 3,000 rpm for 10 min, after which blood sera were dispensed and cryopreserved at -80°C until analysis.

Measurement methods

Serum VEGF-A concentrations were measured using enzyme-linked immunosorbent assay (Canine VEGF Quantikine ELISA kit, R&D Systems Inc., Minneapolis, MN, USA). This assay recognizes natural and recombinant canine VEGF-A. No significant cross-reactivity of other recombinant VEGF family was observed. A microplate reader (Multiskan GO, Thermo Fisher Scientific, Inc., Waltham, MA, USA) was used for absorbance, with measurements made at 450 nm and optical error correction conducted at 540 nm. Results were the average of two wells' worth of measurements for each sample. The calibration curve was created using a four-parameter logistical regression equation.

Statistical analysis

The ages and serum VEGF-A concentrations of dogs in each group were expressed as medians [ranges]. Differences according to sex were determined using a χ^2 test. The Mann–Whitney *U* test was used for comparisons between two groups, the Kruskal–Wallis test for comparisons between three or more groups, and the Dunn method as a post hoc test in cases where statistical significance was determined. The threshold for statistical significance was set at $P < 0.05$. Box-and-whisker plots were used to visualize the distribution comparison of the data belonging to the three or more groups for serum VEGF-A concentration. Box indicates median and interquartile range with whiskers depicting minimum and maximum values for the entire group. All analyses were conducted using GraphPad Prism 9 software (GraphPad Software, Inc., San Diego, CA, USA).

RESULTS

Clinical information

The age of dogs in the proliferative disease group was 11.6 years [3.4–15.7 years], with 83 castrated males, 27 non-castrated males, 81 spayed females, and 11 non-spayed females when sorted by sex. The age of dogs in the healthy canine group was 2.4 years [1.5–2.6 years], with 6 non-castrated males, and 7 non-spayed females when sorted by sex. The proliferative disease canine group was significantly older than the healthy canine group, but no differences according to sex were observed in each group. The clinical information of dogs is shown in [Supplementary Table](#).

Histopathological diagnosis

The histopathological diagnoses of the proliferative diseases were as follows: hepatocellular carcinoma (n=43), hepatocellular adenoma (n=14), hepatic nodular hyperplasia (n=6), adrenal pheochromocytoma (n=30), adrenocortical carcinoma (n=14), adrenocortical adenoma (n=12), colorectal adenocarcinoma (n=20), colorectal adenoma (n=5), inflammatory colorectal polyps (n=15), pulmonary adenocarcinoma (n=21), pulmonary histiocytic sarcoma (n=6), and follicular thyroid carcinoma (n=16).

Serum VEGF-A concentration

A comparative analysis of serum VEGF-A concentration was conducted between the proliferative disease and healthy canine groups (Table 1).

The average serum VEGF-A concentration of dogs with hepatic tumors were significantly higher than that of healthy dogs, but not significantly different from that of dogs with hepatic nodular hyperplasia. The average serum VEGF-A concentration of dogs with colorectal adenocarcinoma and inflammatory colorectal polyp were significantly higher than that of healthy dogs, but not significantly different from that of dogs with colorectal adenoma. The average serum VEGF-A concentrations in dogs with adrenal tumors, pulmonary tumors, and follicular thyroid carcinoma were all significantly higher than that of healthy dogs.

Serum VEGF-A concentrations in tumors according to the site (organ) of occurrence were 28 pg/ml [0–158 pg/ml] for hepatic tumors, 31 pg/ml [0–187 pg/ml] for adrenal tumors, 37 pg/ml [0–111 pg/ml] for colorectal tumors, and 35 pg/ml [0–131 pg/ml] for pulmonary tumors (Fig. 1). These values in these proliferative disease groups were significantly higher than that of the healthy group, but Serum VEGF-A concentrations were not significantly different among each site (organ) of occurrence.

Serum VEGF-A concentrations compared between dogs with benign disease and those with malignant tumors were 34 pg/ml [0–111 pg/ml] for the benign disease group and 35 pg/ml [0–187 pg/ml] for the malignant tumor group (Fig. 2). These values were not significantly different between both groups but were significantly higher than those in the healthy group.

Table 1. Serum vascular endothelial growth factor-A concentrations of proliferative disease canine group and the healthy canine group

Group	Histopathological diagnosis	Median (pg/ml)	Range (pg/ml)	P value*
Healthy control		4	0–21	
Hepatic tumor	Hepatocellular carcinoma	30	0–158	<0.001
	Hepatocellular adenoma	32	0–49	0.003
	Hepatic nodular hyperplasia	18	0–51	0.595
Adrenal tumor	Adrenal pheochromocytoma	32	0–187	<0.001
	Adrenocortical carcinoma	32	3–161	0.002
	Adrenocortical adenoma	27	0–106	0.005
Colorectal tumor	Colorectal adenocarcinoma	36	0–75	0.002
	Colorectal adenoma	43	0–48	0.144
	Inflammatory colorectal polyps	37	0–111	<0.001
Pulmonary tumor	Pulmonary adenocarcinoma	35	0–131	0.002
	Pulmonary histiocytic sarcoma	35	4–107	0.016
Thyroid tumor	Follicular thyroid carcinoma	35	0–106	0.009

*Results compared with healthy control by the Dunn's test.

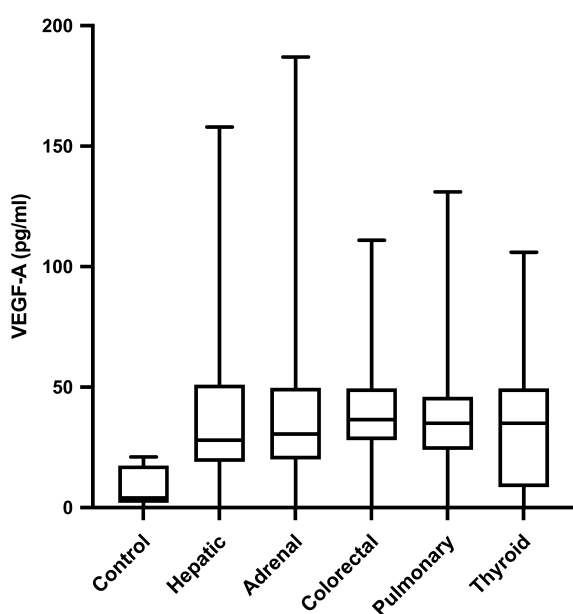


Fig. 1. Box-and-whisker plots depicting serum vascular endothelial growth factor-A concentrations categorized by tumor occurrence site (liver, adrenal gland, colorectal, lung, and thyroid gland).

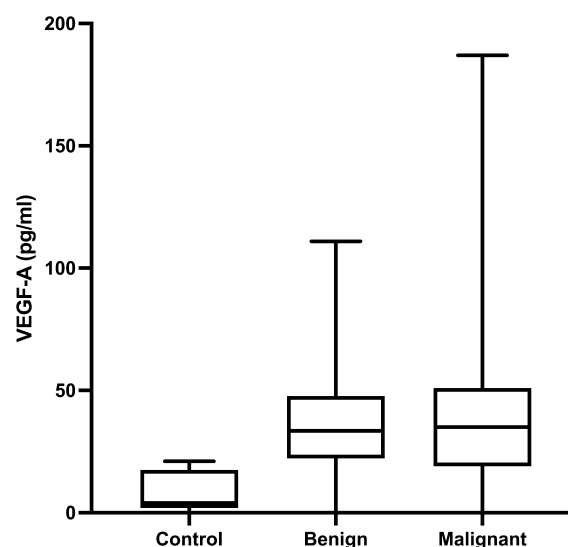


Fig. 2. Box-and-whisker plots depicting serum vascular endothelial growth factor-A concentrations of dogs categorized into a benign disease group and a malignant tumor group.

DISCUSSION

In the present study, serum VEGF-A concentrations were measured in 202 dogs with various proliferative diseases. Overall, serum VEGF-A concentrations tend to be higher in dogs with proliferative diseases compared to normal control dogs. The serum VEGF-A concentrations were significantly higher in dogs with neoplastic lesions compared to healthy dogs, except for colorectal adenoma.

Compared with non-epithelial tumors, epithelial tumors are considered to have high serum VEGF-A concentrations due to their abundant blood vessels. In fact, in canine epithelial tumors, a correlation between VEGF-A expression in tumor tissues and microvessel density have been reported [23, 31, 32].

Needle biopsy sensitivity for hepatic lesions in canines is reportedly 34.8% [3], and identification of hepatic tumors with needle biopsy alone is difficult in many cases. There are virtually no useful biomarkers for the differential diagnosis of hepatic tumors, and diagnosis methods using CT contrast examinations [19] or histopathological diagnoses based on excisional biopsy are necessary, but both must be implemented under anesthesia. Humans with hepatocellular carcinoma have higher serum VEGF-A concentrations relative to patients with benign tumors [42]. Furthermore, the blood VEGF-A concentration of patients with hepatocellular carcinoma is correlated with cancer progression or stage [21, 29], hence it is used as a prognosis indicator. Serum VEGF-A concentrations were also significantly higher in dogs with hepatocellular carcinoma and adenoma relative to healthy dogs in our study, but no significant difference in hepatic nodular hyperplasia was observed between both groups. Therefore, the measurement of serum VEGF-A concentration may be useful for the differentiation between hepatic neoplastic and hyperplastic lesions in dogs.

Although no clear differences in serum VEGF-A concentration according to histopathological diagnoses were observed in the adrenal tumor group, the recorded serum VEGF-A concentrations in all groups were significantly higher than those in the healthy canine group. The results suggest that serum VEGF-A concentration cannot be used to distinguish different types of adrenal tumors, such as pheochromocytoma, adrenocortical adenoma and carcinoma. However, molecular-targeted therapy, which targets VEGF-A, can be useful regardless of histopathological diagnosis in adrenal tumor cases.

The colorectal tumor group in this study included many cases of inflammatory polyps, and significantly higher serum VEGF-A concentrations were observed with inflammatory polyps and adenocarcinomas relative to the healthy canine group. Medical reports have indicated a correlation between inflammatory diseases and VEGF [11]. Furthermore, inflammatory colorectal polyp of miniature dachshund has been reported to show severe vascularization [40], and may develop concurrent lesions of adenoma and adenocarcinoma [14, 33]. The present study results suggest that molecular VEGF-A-targeted therapy can be useful in canine colorectal tumors. Additionally, similar to hepatic tumors, the measurement of serum VEGF-A concentration may support clinical diagnosis of colorectal adenocarcinoma and inflammatory colorectal polyps since no significant differences were observed in serum VEGF-A concentration between dogs with colorectal adenoma and healthy dogs.

Pulmonary adenocarcinoma in dogs is thought to correspond to non-small cell lung cancer in humans [36], for which anti-VEGF antibody treatment is an indication. Since pulmonary adenocarcinoma had high serum VEGF-A concentrations in the present study, anti-VEGF-antibody-based molecular-targeted therapy may be effective for treatment, just as it is in humans. Additionally, target diseases of anti-VEGF-antibody-based molecular-targeted therapy in the medical fields are all epithelial tumors, and interestingly, reports from veterinary fields have indicated that serum VEGF concentrations are high in non-epithelial tumors such as angiosarcomas [6, 12] and lymphomas [13]. Furthermore, given the novel result of high serum VEGF-A concentrations in pulmonary histiocytic sarcomas in the present study, further analyses may show that non-epithelial tumors can be targeted as well.

Surgical treatment or radiation therapy is chosen for follicular thyroid carcinoma in dogs, depending on the invasion of critical structures in the neck [22]. Additionally, the partial remission rate is 30–50% in cases where chemotherapy is used when distant metastasis has occurred or when surgical treatment and radiation therapy cannot be chosen [22]. Therefore, highly effective chemotherapy regimens against follicular thyroid carcinoma in dogs have been sought, and given the high serum VEGF-A concentrations observed in canine follicular thyroid carcinomas in the present study, similar to other proliferative diseases, molecular VEGF-A-targeted therapy may be a suitable form of treatment.

The present study showed that serum VEGF-A concentrations were significantly higher in dogs with hepatic, adrenal, colorectal, and pulmonary tumors, as well as follicular thyroid carcinomas, relative to healthy canines. No reports have indicated the serum VEGF-A concentrations of these proliferative diseases in dogs; we present novel study on high serum VEGF-A concentrations observed in these cases. Furthermore, the present study results suggest that the measurement of serum VEGF-A concentration in hepatic and colorectal tumors can be useful for differential diagnosis.

There were several limitations in this study. Serum VEGF-A concentrations in dogs with proliferative disease were only evaluated at one time point, and postoperative changes were not examined. In addition, expression of VEGF receptor was not concurrently investigated. Therefore, the functional association between the proliferative lesions and serum VEGF-A was not confirmed. Another limitation was that the healthy control group was comprised of dogs of a same breed (i.e. beagle) that were significantly younger than the proliferative disease group, which might result in age and breed biases.

Further studies on the role of VEGF-A for tumor proliferation and its metastasis mechanisms are required to pursue the potential of anti-angiogenesis therapy using VEGF-A. In addition, a future large-scale study on the relationship between VEGF-A and individual tumors are necessary to identify malignant tumors which targeted by the anti-angiogenesis therapy in dogs. The present study suggests that angiogenesis-inhibiting therapy based on molecular-targeted therapy, which targets VEGF-A, can be applied in clinical settings.

CONFLICTS OF INTEREST. The authors declare no conflict of interest related to this study.

REFERENCES

- Adams, J., Carder, P. J., Downey, S., Forbes, M. A., MacLennan, K., Allgar, V., Kaufman, S., Hallam, S., Bicknell, R., Walker, J. J., Cairnduff, F., Selby, P. J., Perren, T. J., Lansdown, M. and Banks, R. E. 2000. Vascular endothelial growth factor (VEGF) in breast cancer: comparison of plasma, serum, and tissue VEGF and microvessel density and effects of tamoxifen. *Cancer Res.* **60**: 2898–2905. [Medline]
- Argyle, D. J. and Khanna, C. 2013. Tumor biology and metastasis. pp. 30–50. In: Withrow and McEwen's Small Animal Clinical Oncology, 5th ed. (Withrow, S. J., Vail, D. M. and Page, R. L., eds.), Elsevier Saunders, St. Louis.
- Bahr, K. L., Sharkey, L. C., Murakami, T. and Feeney, D. A. 2013. Accuracy of US-guided FNA of focal liver lesions in dogs: 140 cases (2005–2008). *J. Am. Anim. Hosp. Assoc.* **49**: 190–196. [Medline] [CrossRef]
- Beştaş, R., Kaplan, M. A. and Işıkdoğan, A. 2014. The correlation between serum VEGF levels and known prognostic risk factors in colorectal carcinoma. *Hepatogastroenterology* **61**: 267–271. [Medline]
- Clifford, C. A., Hughes, D., Beal, M. W., Henry, C. J., Drobotz, K. J. and Sorenmo, K. U. 2002. Vascular endothelial growth factor concentrations in body cavity effusions in dogs. *J. Vet. Intern. Med.* **16**: 164–168. [Medline] [CrossRef]
- Clifford, C. A., Hughes, D., Beal, M. W., Mackin, A. J., Henry, C. J., Shofer, F. S. and Sorenmo, K. U. 2001. Plasma vascular endothelial growth factor concentrations in healthy dogs and dogs with hemangiosarcoma. *J. Vet. Intern. Med.* **15**: 131–135. [Medline] [CrossRef]
- Cunningham, D., Lang, I., Marcuello, E., Lorusso, V., Ocvirk, J., Shin, D. B., Jonker, D., Osborne, S., Andre, N., Waterkamp, D., Saunders M. P., AVEX study investigators. 2013. Bevacizumab plus capecitabine versus capecitabine alone in elderly patients with previously untreated metastatic colorectal cancer (AVEX): an open-label, randomised phase 3 trial. *Lancet Oncol.* **14**: 1077–1085. [Medline] [CrossRef]
- de Queiroz, G. F., Dagli, M. L., Meira, S. A. and Matera, J. M. 2013. Serum vascular endothelial growth factor in dogs with soft tissue sarcomas. *Vet. Comp. Oncol.* **11**: 230–235. [Medline] [CrossRef]
- Ferrari, G. and Scagliotti, G. V. 1996. Serum and urinary vascular endothelial growth factor levels in non-small cell lung cancer patients. *Eur. J. Cancer* **32A**: 2368–2369. [Medline] [CrossRef]
- Folkman, J. 1971. Tumor angiogenesis: therapeutic implications. *N. Engl. J. Med.* **285**: 1182–1186. [Medline] [CrossRef]
- Folkman, J. 1995. Angiogenesis in cancer, vascular, rheumatoid and other disease. *Nat. Med.* **1**: 27–31. [Medline] [CrossRef]
- Frenz, M., Kaup, F. J. and Neumann, S. 2014. Serum vascular endothelial growth factor in dogs with haemangiosarcoma and haematoma. *Res. Vet. Sci.* **97**: 257–262. [Medline] [CrossRef]
- Gentilini, F., Calzolari, C., Turba, M. E., Agnoli, C., Fava, D., Forni, M. and Bergamini, P. F. 2005. Prognostic value of serum vascular endothelial growth factor (VEGF) and plasma activity of matrix metalloproteinase (MMP) 2 and 9 in lymphoma-affected dogs. *Leuk. Res.* **29**: 1263–1269. [Medline] [CrossRef]
- Horikirizono, H., Ishigaki, K., Amaha, T., Iizuka, K., Nagumo, T., Tamura, K., Seki, M., Edamura, K., Watari, T. and Asano, K. 2019. Clinical features and surgical treatment of inflammatory colorectal polyps in miniature dachshunds: 40 cases (2002–2015). *Jpn. J. Vet. Res.* **67**: 173–183.
- Hurwitz, H., Fehrenbacher, L., Novotny, W., Cartwright, T., Hainsworth, J., Heim, W., Berlin, J., Baron, A., Griffing, S., Holmgren, E., Ferrara, N., Fyfe, G., Rogers, B., Ross, R. and Kabbinavar, F. 2004. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N. Engl. J. Med.* **350**: 2335–2342. [Medline] [CrossRef]
- Jinno, K., Tanimizu, M., Hyodo, I., Nishikawa, Y., Hosokawa, Y., Doi, T., Endo, H., Yamashita, T. and Okada, Y. 1998. Circulating vascular endothelial growth factor (VEGF) is a possible tumor marker for metastasis in human hepatocellular carcinoma. *J. Gastroenterol.* **33**: 376–382. [Medline] [CrossRef]
- Kato, Y., Asano, K., Mogi, T., Kutara, K., Teshima, K., Edamura, K., Tsumagari, S., Hasegawa, A. and Tanaka, S. 2007. Clinical significance of circulating vascular endothelial growth factor in dogs with mammary gland tumors. *J. Vet. Med. Sci.* **69**: 77–80. [Medline] [CrossRef]
- Kumar, H., Heer, K., Lee, P. W., Duthie, G. S., MacDonald, A. W., Greenman, J., Kerin, M. J. and Monson, J. R. 1998. Preoperative serum vascular endothelial growth factor can predict stage in colorectal cancer. *Clin. Cancer Res.* **4**: 1279–1285. [Medline]
- Kutara, K., Seki, M., Ishikawa, C., Sakai, M., Kagawa, Y., Iida, G., Ishigaki, K., Teshima, K., Edamura, K., Nakayama, T. and Asano, K. 2014. Triple-phase helical computed tomography in dogs with hepatic masses. *Vet. Radiol. Ultrasound* **55**: 7–15. [Medline] [CrossRef]
- Kwon, K. A., Kim, S. H., Oh, S. Y., Lee, S., Han, J. Y., Kim, K. H., Goh, R. Y., Choi, H. J., Park, K. J., Roh, M. S., Kim, H. J., Kwon, H. C. and Lee, J. H. 2010. Clinical significance of preoperative serum vascular endothelial growth factor, interleukin-6, and C-reactive protein level in colorectal cancer. *BMC Cancer* **10**: 203. [Medline] [CrossRef]
- Li, X., Feng, G. S., Zheng, C. S., Zhuo, C. K. and Liu, X. 2004. Expression of plasma vascular endothelial growth factor in patients with hepatocellular carcinoma and effect of transcatheter arterial chemoembolization therapy on plasma vascular endothelial growth factor level. *World J. Gastroenterol.* **10**: 2878–2882. [Medline] [CrossRef]
- Lunn, K. F. and Page, R. L. 2013. Tumors of the endocrine system. pp. 504–531. In: Withrow and McEwen's Small Animal Clinical Oncology, 5th ed. (Withrow, S. J., Vail, D. M. and Page, R. L., eds.), Elsevier Saunders, St. Louis.
- Maiolino, P., De Vico, G. and Restucci, B. 2000. Expression of vascular endothelial growth factor in basal cell tumours and in squamous cell carcinomas of canine skin. *J. Comp. Pathol.* **123**: 141–145. [Medline] [CrossRef]
- Manders, P., Sweep, F. C., Tjan-Heijnen, V. C., Geurts-Moespot, A., van Tienoven, D. T., Foekens, J. A., Span, P. N., Bussink, J. and Beex, L. V. 2003. Vascular endothelial growth factor independently predicts the efficacy of postoperative radiotherapy in node-negative breast cancer patients. *Clin. Cancer Res.* **9**: 6363–6370. [Medline]
- Michishita, M., Uto, T., Nakazawa, R., Yoshimura, H., Ogihara, K., Naya, Y., Tajima, T., Azakami, D., Kishikawa, S., Arai, T. and Takahashi, K. 2013. Antitumor effect of bevacizumab in a xenograft model of canine hemangiopericytoma. *J. Pharmacol. Sci.* **121**: 339–342. [Medline] [CrossRef]
- Mohammed, S. I., Bennett, P. F., Craig, B. A., Glickman, N. W., Mutsaers, A. J., Snyder, P. W., Widmer, W. R., DeGortari, A. E., Bonney, P. L. and Knapp, D. W. 2002. Effects of the cyclooxygenase inhibitor, piroxicam, on tumor response, apoptosis, and angiogenesis in a canine model of human invasive urinary bladder cancer. *Cancer Res.* **62**: 356–358. [Medline]
- Mukozu, T., Nagai, H., Matsui, D., Kanekawa, T. and Sumino, Y. 2013. Serum VEGF as a tumor marker in patients with HCV-related liver cirrhosis and hepatocellular carcinoma. *Anticancer Res.* **33**: 1013–1021. [Medline]
- Mustonen, T. and Alitalo, K. 1995. Endothelial receptor tyrosine kinases involved in angiogenesis. *J. Cell Biol.* **129**: 895–898. [Medline] [CrossRef]

29. Poon, R. T., Lau, C. P., Cheung, S. T., Yu, W. C. and Fan, S. T. 2003. Quantitative correlation of serum levels and tumor expression of vascular endothelial growth factor in patients with hepatocellular carcinoma. *Cancer Res.* **63**: 3121–3126. [[Medline](#)]
30. Restucci, B., Borzacchiello, G., Maiolino, P., Martano, M., Paciello, O. and Papparella, S. 2004. Expression of vascular endothelial growth factor receptor Flk-1 in canine mammary tumours. *J. Comp. Pathol.* **130**: 99–104. [[Medline](#)] [[CrossRef](#)]
31. Restucci, B., Maiolino, P., Paciello, O., Martano, M., De Vico, G. and Papparella, S. 2003. Evaluation of angiogenesis in canine seminomas by quantitative immunohistochemistry. *J. Comp. Pathol.* **128**: 252–259. [[Medline](#)] [[CrossRef](#)]
32. Restucci, B., Papparella, S., Maiolino, P. and De Vico, G. 2002. Expression of vascular endothelial growth factor in canine mammary tumors. *Vet. Pathol.* **39**: 488–493. [[Medline](#)] [[CrossRef](#)]
33. Saito, T., Chambers, J. K., Nakashima, K., Uchida, E., Ohno, K., Tsujimoto, H., Uchida, K. and Nakayama, H. 2018. Histopathologic features of colorectal adenoma and adenocarcinoma developing within inflammatory polyps in Miniature Dachshunds. *Vet. Pathol.* **55**: 654–662. [[Medline](#)] [[CrossRef](#)]
34. Saltz, L. B., Clarke, S., Díaz-Rubio, E., Scheithauer, W., Figer, A., Wong, R., Koski, S., Lichinitser, M., Yang, T. S., Rivera, F., Couture, F., Sirzén, F. and Cassidy, J. 2008. Bevacizumab in combination with oxaliplatin-based chemotherapy as first-line therapy in metastatic colorectal cancer: a randomized phase III study. *J. Clin. Oncol.* **26**: 2013–2019. [[Medline](#)] [[CrossRef](#)]
35. Salven, P., Teerenhovi, L. and Joensuu, H. 1997. A high pretreatment serum vascular endothelial growth factor concentration is associated with poor outcome in non-Hodgkin's lymphoma. *Blood* **90**: 3167–3172. [[Medline](#)] [[CrossRef](#)]
36. Sandler, A., Gray, R., Perry, M. C., Brahmer, J., Schiller, J. H., Dowlati, A., Lilienbaum, R. and Johnson, D. H. 2006. Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. *N. Engl. J. Med.* **355**: 2542–2550. [[Medline](#)] [[CrossRef](#)]
37. Scharf, V. F., Farese, J. P., Coomer, A. R., Milner, R. J., Taylor, D. P., Salute, M. E., Chang, M. N., Neal, D. and Siemann, D. W. 2013. Effect of bevacizumab on angiogenesis and growth of canine osteosarcoma cells xenografted in athymic mice. *Am. J. Vet. Res.* **74**: 771–778. [[Medline](#)] [[CrossRef](#)]
38. Scheidegger, P., Weiglhofer, W., Suarez, S., Kaser-Hotz, B., Steiner, R., Ballmer-Hofer, K. and Jaussi, R. 1999. Vascular endothelial growth factor (VEGF) and its receptors in tumor-bearing dogs. *Biol. Chem.* **380**: 1449–1454. [[Medline](#)] [[CrossRef](#)]
39. Sharif, M. R., Shaabani, A., Mahmoudi, H., Nikouejad, H., Akbari, H. and Einollahi, B. 2014. Association of the serum vascular endothelial growth factor levels with benign prostate hyperplasia and prostate malignancies. *Nephrourol. Mon.* **6**: e14778. [[Medline](#)] [[CrossRef](#)]
40. Uchida, E., Chambers, J. K., Nakashima, K., Saito, T., Ohno, K., Tsujimoto, H., Nakayama, H. and Uchida, K. 2016. Pathologic features of colorectal inflammatory polyps in Miniature Dachshunds. *Vet. Pathol.* **53**: 833–839. [[Medline](#)] [[CrossRef](#)]
41. Werther, K., Christensen, I. J., Nielsen H. J., Danish RANX05 Colorectal Cancer Study Group. 2002. Prognostic impact of matched preoperative plasma and serum VEGF in patients with primary colorectal carcinoma. *Br. J. Cancer* **86**: 417–423. [[Medline](#)] [[CrossRef](#)]
42. Zhao, J., Hu, J., Cai, J., Yang, X. and Yang, Z. 2003. Vascular endothelial growth factor expression in serum of patients with hepatocellular carcinoma. *Chin. Med. J. (Engl.)* **116**: 772–776. [[Medline](#)]