



Lymphovascular Invasion
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Introduction

Lymphovascular invasion (LVI) is the process of neoplastic cells gaining access to lymphatics or blood vessels and is recognized as a criterion of malignancy for many human and animal tumors. However, the precise histological features used to document this process are not well described in veterinary pathology. Although lymphovascular

invasion is typically included in tumor descriptions, the criteria used to make this interpretation are often lacking.

This guideline provides criteria used to differentiate true lymphovascular invasion from pseudo-vascular invasion (Notes A, B) or artifactual displacement of neoplastic cells into vascular lumens. Additionally, it lists the histologic features which should be described when vascular invasion is reported. Furthermore recommendations for future studies are provided, in particular, lymphatic vs blood vessel LVI, intratumoral vs peritumoral LVI and a potential scoring system for LVI. While these criteria have been reported to be important for assessment of human tumors, their importance in animal tumors has not yet been established.

Lymphovascular Invasion:

Histopathologic criteria

Presence or absence of lymphovascular invasion by tumors must be reported together with criteria used to define LVI. At least one of the following criteria is required to verify LVI¹⁻⁶

1. Thrombus adherent to intravascular tumor
2. Tumor cells invading through a vessel wall and endothelium
3. Neoplastic cells within a space lined by lymphatic or blood vascular endothelium
4. Neoplastic cells in a structure confirmed to be a lymphatic or blood vessel by the use of immunohistochemical markers (Note C)

Lymphovascular invasion must be distinguished from pseudo-vascular invasion and retraction artifact (**Figures 1-5**). (Note B)

Reporting format:

Lymphovascular Invasion (report format below)

_____ Not identified

_____ Equivocal (Notes A,B)

_____ Present

Criteria used to determine lymphovascular invasion¹⁻⁶

____ Thrombus adherent to intravascular tumor

____ Tumor cells invading through a vessel wall and endothelium

____ Neoplastic cells within a space lined by lymphatic or blood vascular endothelium

____ Neoplastic cells in a structure that has been confirmed to be a lymphatic or blood vessel using immunohistochemistry (Note C)

Number of LVI foci (within a minimum of one representative section of tumor and peritumoral tissue. Report the number of foci of LVI within all sections examined.)

____ Few (< 5 foci)

____ Moderate (5 – 10 foci)

____ Many (> 10 foci)

Type of vessels invaded

____ Muscular wall evident

____ No muscular wall evident

Site of lymphovascular invasion

____ Intratumoral (number of LVI foci)

____ Peritumoral (number of LVI foci)

Discussion

Although LVI is recognized as a marker of tumor malignancy, suggesting aggressive biological behavior and increased probability of metastatic disease in a number of different human cancers;^{1,2,7,8,5,9,10} in veterinary medicine, this parameter has only been extensively evaluated in canine and feline mammary tumors.¹¹⁻²⁰

A study of human thyroid tumors reported that the type of LVI (ie blood versus lymphatic) was more important than the number of LVI to predict metastases.^{2,4} Neither the scoring of LVI nor the type of LVI have been evaluated in animal tumors and both should be considered. Studies of human breast and colorectal cancer suggest blood vascular invasion, in contrast to lymphatic invasion, is an independent indicator of

aggressive behavior and poor prognostic indicator.^{7,8} Similarly, in human thyroid carcinomas, the presence of definitive blood vascular invasion is associated with more aggressive behavior and metastasis.^{2,5} Typically, the criteria for identification of lymphovascular invasion are not defined or standardized in veterinary pathology. In contrast, criteria defining lymphatic and blood vascular invasion, as well as which criteria are more reliable, have been proposed and adopted for human cancer assessment, including breast, thyroid and endometrial carcinomas.^{21,22,4,5,23,24}

The most rigorous criteria used to determine lymphovascular invasion in human cancers are the presence of a thrombus adherent to intravascular tumor or invasion of tumor cells through vessel walls and endothelium. The finding of a thrombus (i.e. fibrin, platelets, etc) adherent to tumor cells within a vascular space is more definitive than the presence of tumor cells alone within a vascular space as the latter could be pseudo-vascular invasion. The use of strict criteria for blood vascular invasion was correlated with more accurate prognoses in a review of thyroid carcinomas in humans. When rigid criteria such as tumor thrombus or tumor in the wall of a vessel were applied to cases of human thyroid carcinoma only 118 (3%) of 4000 cases had these types of LVI, however, 35% of these carcinomas developed distant metastases.⁵ In another study, using less rigid criteria for LVI, 47% of cases had LVI but only 8% of the cases developed distant metastases.²⁵ The presence of neoplastic cells within a space lined by endothelial cells immunopositive for blood or lymphatic vascular markers is less definitive evidence of true vascular invasion and could reflect artifactual displacement of neoplastic cells in vessels (Fig 4,5).^{1-5,9,10,6} Given the lack of studies and lack of defined criteria for LVI or pseudo-vascular invasion, the likelihood of overdiagnosis of LVI in animal tumors is great. When tumor cells are found within the lumen of vascular structures, it is prudent to search for the more definitive features of LVI and to ascertain if the tumor is in blood vessels or lymphatics. The authors are not aware of any veterinary studies that distinguished the more rigorous criteria from less definitive criteria to confirm LVI and correlated each with outcome assessments to know which, if any, predicted metastases.

Small blood vessels lacking a muscular wall cannot be reliably distinguished from lymphatics in routinely stained sections, necessitating use of immunohistochemical markers to make the distinction (Fig 4). A number of immunohistochemical markers have been used to identify endothelial cells in blood and lymphatic vessels in both humans and animals, including CD31 and Factor VIII related antigen which bind both lymphatic and blood vascular endothelium and prospero-related homeobox gene-1 (PROX-1), D2-40 and lymphatic vessel endothelial receptor 1 (LYVE-1) which are reported to be specific for lymphatic endothelium.^{26,3,27-31} Their utility in animal tumors requires validation although one study of lymphatic and blood vessels in canine mammary tumors found Prox-1 and CD 31 as the preferred antibodies for identifying lymphatic and blood vessels respectively.²⁹ The prognostic significance of lymphatic versus blood vascular invasion has not been evaluated in animal studies.

Lymphovascular invasion (Figs 1-2) must be distinguished from stromal retraction artifact (Fig 5) and from pseudo-vascular invasion. Retraction artifact forms an artifactual space which can surround tumor foci and can be distinguished from intravascular neoplasia by the absence of an endothelial cell lining. Retraction artifact is commonly seen in many epithelial tumors in which tumor cells retract from surrounding stroma. Circumanal gland tumors often display this artifact (Fig 5). Pseudo-vascular invasion is the presence of neoplastic cells within vascular spaces, but the cells are not present as a result of tumor invasion of vessels. Displacement of neoplastic cells into vessels secondary to manipulation of the neoplasm at the time of biopsy, surgical excision, grossing procedure or tissue sectioning (ie, "floaters") can result in this phenomenon.^{21,22,5,23,24} Physical manipulation of the thyroid gland with non-neoplastic lesions also produces pseudo-vascular emboli similar to iatrogenic introduction of neoplastic cells into vessels with manipulation of uterine specimens during robotic surgery of uterine specimens in humans.^{1,2,5,9}

The phenomenon of pseudo-vascular invasion has not been evaluated in veterinary cancer case studies. A study of canine cutaneous plasmacytomas reported that 16% of 125 dogs had intravascular tumor emboli but there was no association with metastases and their presence did not affect prognosis.³² In the discussion, the authors

commented that displacement of tumor cells by pressure or trauma could account for the reported findings. Intravascular neoplastic cells were more common if the tumor was on the distal limb.³² This study with a tumor considered to be “benign” in dogs highlights the need to correlate accepted events of aggressiveness / malignancy with outcome assessments as well as document the type of evidence for LVI. In this study the authors reported emboli but more rigorous criteria of invasion such as fibrin adhered to intravascular tumor or tumor cells invading the wall and endothelium of a vessel not reported (Figs 3, 4). Was the event trauma- induced pseudoinvasion or was this true invasion but the tumor biology was such that neoplastic cells would remain intravascular and not metastasize? How thorough were outcome assessments and were cases followed long enough for metastases to develop? Were tumor cells in lymphatics or blood vessels?

The presence of subendothelial neoplastic cells protruding into a vascular lumen can be difficult to interpret. In this circumstance, neoplastic cells may not have invaded through the endothelium representing vascular impingement, not LVI. Alternatively, these findings could occur in a neoplasm which has invaded through the vascular endothelium but subsequently has been re-endothelialized with a layer of endothelial cells on the luminal surface of the neoplasm and, as such, has been included as a criterion of LVI in some manuscripts.⁵ Identification of subendothelial tumor cells warrants further evaluation for more strict criteria of vascular invasion, such as a thrombus adherent to intravascular tumor or foci of tumor cells invading both vessel wall and endothelium.

Studies of human breast, thyroid and prostate cancer show widespread metastases more commonly associated with blood vascular invasion in contrast to lymphatic invasion.^{7,8,5} Animal tumors may show similar distinctions between blood and lymphatic vascular invasion, warranting detailed descriptions of the type of vessels invaded. To assess this, reported vascular invasion must detail the types of vessels involved (ie, if a muscular wall can be identified as a blood vascular channel or if only an endothelial lining is seen). Immunohistochemical markers specific for lymphatic and

blood vessels are needed to determine the type of vascular invasion if tumor is restricted to vessels with only a thin endothelial lining.

Three-tiered scoring of the extent of LVI (absence, focal and substantial) has been utilized in evaluations of human endometrial carcinoma and, although there was good reproducibility of identifying presence or absence of LVI, there was varied reproducibility regarding LVI extent. Scoring of LVI has not been addressed in veterinary studies and should be considered in future investigations.

A thorough reassessment of LVI is sorely needed in veterinary oncology with attention to the specific details described in this guideline and under future considerations. These studies should consider specifics of tumoral vs peritumoral lymphatics and blood vessels and correlation to lymph node and distant metastases. The significance of LVI in relation to specific tumor types must be determined.

LVI has been correlated with intratumoral and peritumoral lymphovascular density (LVD) in a number of human tumors.³³⁻³⁶ LVD is an enumeration of lymphatics within a defined area of a tumor and is used as an indicator of lymphangiogenesis and therefore probable lymph node metastasis. Both LVD and LVI are used as predictors of lymph node metastases in human breast cancer and peritumoral lymphatic vessels may be the main route for dissemination of the tumor.^{35,36} Peritumoral LVD is more closely associated with LVI and nodal metastases than intratumoral LVD in human breast carcinoma studies. Authors suggested that peritumoral lymphatic vessels are the main route to disseminate breast tumor cells.³⁶ Peritumoral LVD and LVI is associated with a poor prognosis in human breast and gastric carcinoma.³³⁻³⁶

Intratumoral microvascular density (IMD), the quantitation of blood vessels (number/mm²) in or around tumors, has been used as an indicator of angiogenesis and by extension LVI and the ability of a tumor to metastasize. New blood vessels in a tumor are required for tumors to grow beyond several millimeters and they are believed to facilitate metastasis, and are associated with more aggressive neoplasms in humans and animals. IMD has been evaluated in a number of animal tumor types³⁷⁻⁴⁵ although correlations with vascular invasion and assessment of peritumoral vascular density was only assessed in one study of canine mammary tumors.⁴⁴ In general, IMD was

associated with either higher grade neoplasms or neoplasms with more malignant histological features (canine mammary gland tumors,⁴⁴ canine seminomas,⁴³ canine cutaneous squamous cell carcinoma,³⁹ and canine cutaneous mast cell tumors⁴¹) Future veterinary studies of microvascular density should include assessment of both lymphatics and blood vessels, evaluation of both intratumoral and peritumoral vascular density (enumeration of vessels in defined area per mm²) and correlate with LVI, nodal and systemic metastases, tumor grade and outcomes. Computational pathology may aid studies that enumerate blood and lymphatic vessels.

Notes

- A. Tissue retraction can form a space which may surround tumor cells mimicking tumor in a lymphovascular space. Lack of demonstrable endothelial lining distinguishes retraction artifact from intravascular neoplasia. Retraction artifact is common in circumanal gland tumors, thyroid tumors and some mammary gland tumors. (See Discussion and Note B).
- B. It can be difficult to distinguish true vascular invasion from pseudo-vascular invasion. The more stringent criteria for defining vascular invasion are demonstration of invasion of the vascular wall by neoplastic cells or presence of neoplastic cells within a thrombus adherent to the vascular wall. In the absence of these findings, the presence of intravascular neoplastic cells could represent either true lymphovascular invasion or pseudo-vascular invasion. This dilemma is apparent in a recent publication reporting lymphatic invasion in oral melanomas in dogs in which intravascular neoplasia is shown within a space which appears to be lined in part by neoplastic cells and cannot be readily confirmed as a lymphatic or blood vascular space.⁴⁶
- C. A variety of immunohistochemical markers have been used to identify endothelial cells in blood and lymphatic vascular channels in humans and in animals.^{26,3,27-31} Some markers such as CD 31 and Factor VIII related antigen do not discriminate between lymphatic and blood vascular endothelium whereas others, such as Lymphatic vessel endothelial receptor 1 (LYVE-1), D2-40 and

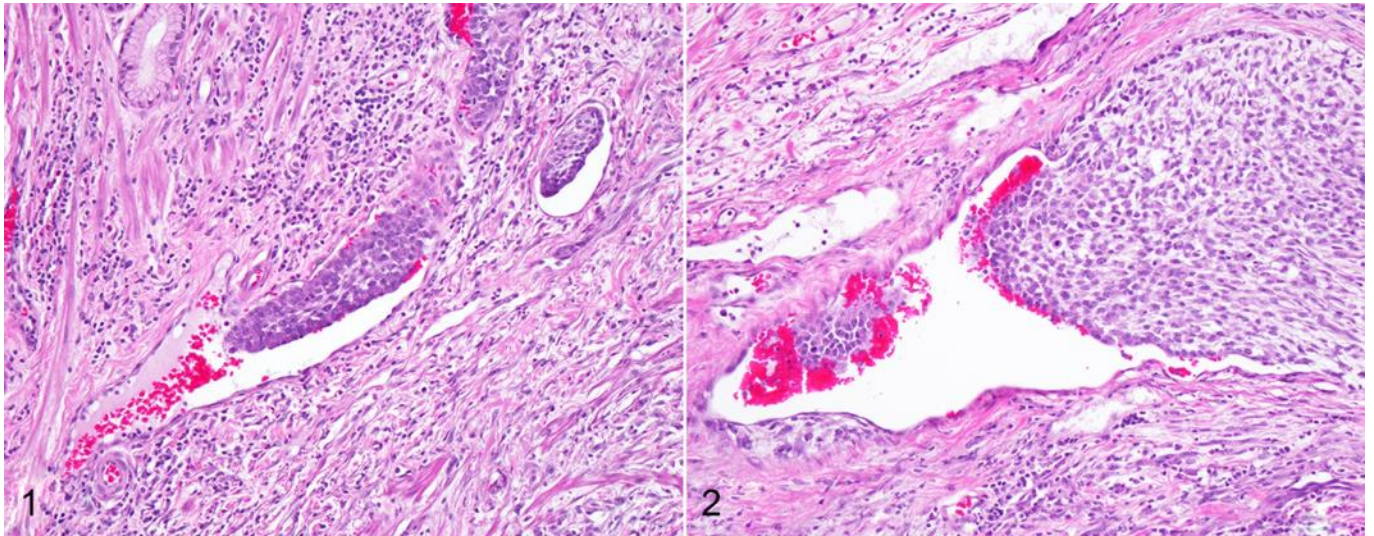
prospero – related homeobox gene-1 (PROX-1) are specific for lymphatic endothelium.^{27,29,31} Use of IHC endothelial markers has been shown to facilitate identification of LVI in tumors in humans,^{22,47} and in mammary and plasma cell tumors in dogs.^{32,29}

Future Considerations:

1. Define criteria to distinguish LVI from pseudo-vascular invasion
 - a. Determine if neoplastic cells bulging into vascular lumen (which could represent impingement or a stage of LVI) is associated with other evidence of LVI or has prognostic importance
2. Assess significance of tumoral vs peritumoral LVI and LVD
3. Evaluate utility of LVI and LVD scoring system
4. Evaluate lymphatic versus blood vascular invasion and determine prognostic significance
 - a. Evaluate tumoral vs peritumoral lymphovascular density
 - i. Develop reporting format and scoring system with enumeration of vessels per defined area in mm²
 - b. Evaluate prognostic significance of intratumoral and peritumoral LVI and LVD
 - i. Consider applications of computational pathology in assessing LVD associated LVI
 - ii. Studies should compare methods of enumeration of LVD: subjective/manual estimates by pathologists versus morphometry, IHC labelled lymphatics and CPATH
 - iii. Studies should compare prognostic significance of intratumoral vs peritumoral LVD by correlating with presence/absence of metastasis and outcome assessments
 - c. Include nodal status, distant metastases and outcome assessments in studies of prognostic significance of LVI for specific tumor types
 - d. Confirm metastases by cytology or histopathology in studies of LVI

i. Recommend use of autopsy data in 10 – 20% cases if possible

Figures:



Figures 1-2: Lymphovascular invasion in an intestinal sarcoma. Neoplastic emboli are present in multiple vascular structures identified by endothelial lining, some of which contain intraluminal red blood cells. This tumor appears to favor blood vessels vs lymphatics. Hepatic metastases were documented.

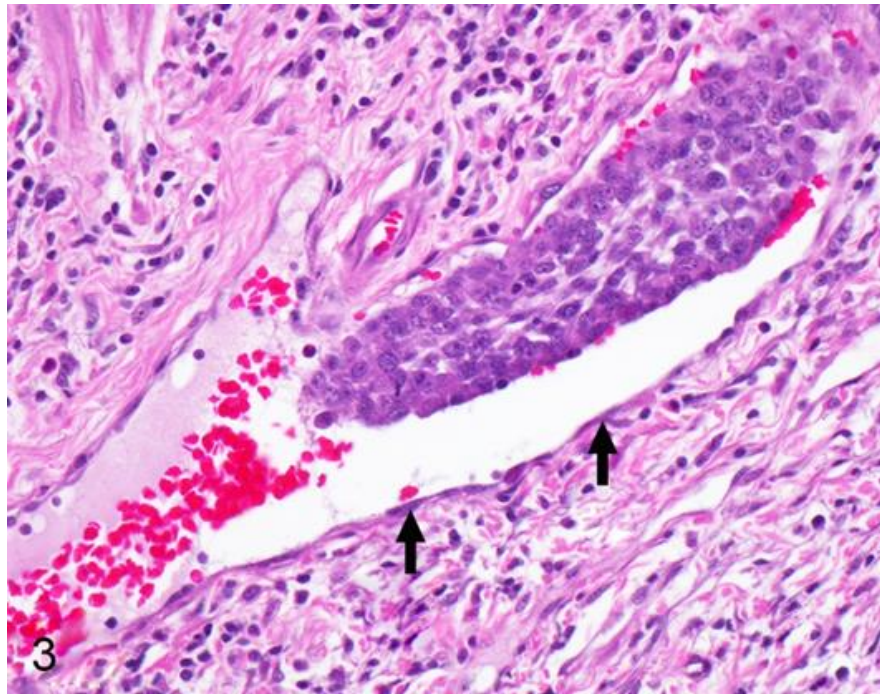


Figure 3: Higher magnification of lymphovascular invasion in an intestinal sarcoma. Endothelial cells are clearly visible (arrows). Metastatic foci were documented in the liver.

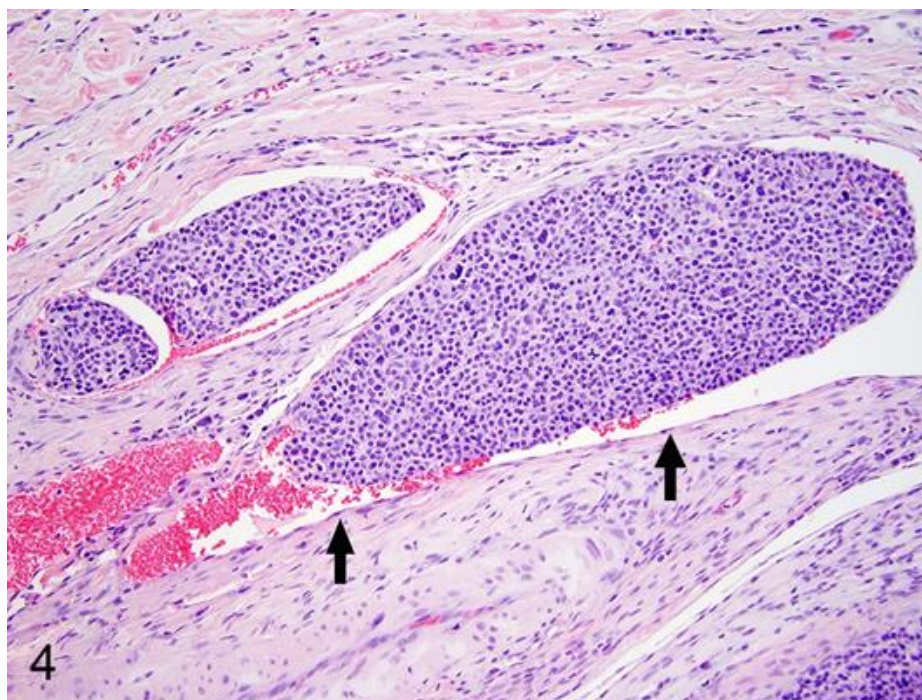


Figure 4: Two intravascular aggregates of neoplastic plasma cells. Arrows illustrate lining endothelial cells with no attached tumor or presence of thrombus. A study of 125 dogs with plasma cell tumors revealed 16% with intravascular neoplastic cells which did not appear to affect the prognosis or result in metastases. (Image courtesy of Gordon Ehrensing DVM, DACVP, Antech Diagnostics)

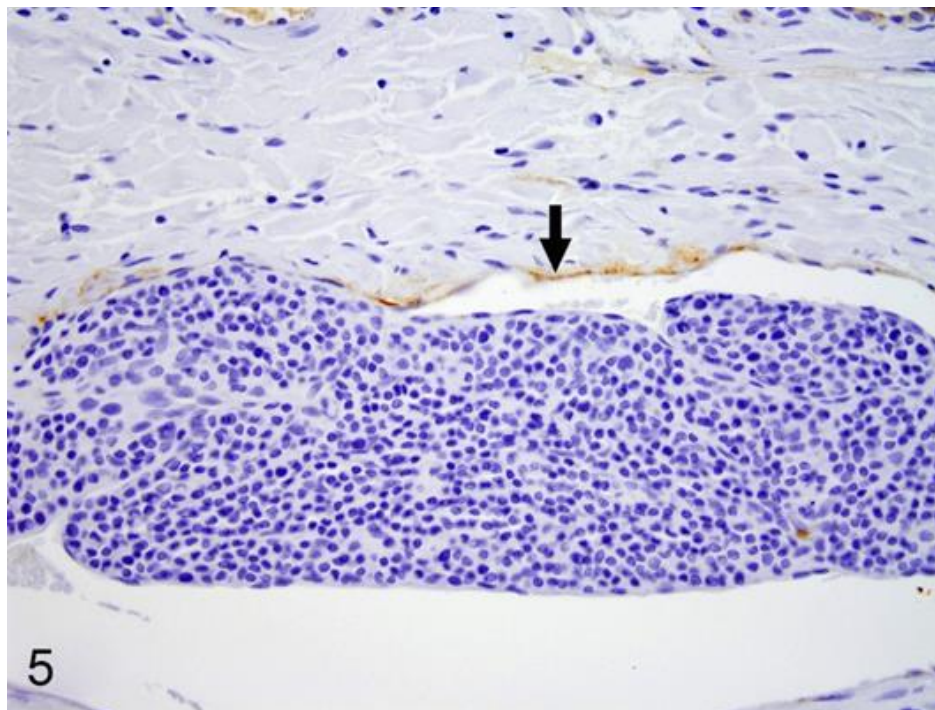


Figure 5: Intravascular aggregate of neoplastic plasma cells. Immunohistochemistry for von Willebrand factor (factor VIII related antigen) shows cytoplasmic immunoreactivity in endothelial cells (arrow), confirming that the cells are within a vessel. This marker does not differentiate between veins and lymphatics. Tumor cells inside the vessel could be due to invasion, pseudoinvasion (displacement) or bulging/impingement. The latter was deemed less likely as none of the emboli were covered by endothelial cells. (Image courtesy of Gordon Ehrensing DVM, DACVP, Antech Diagnostics)

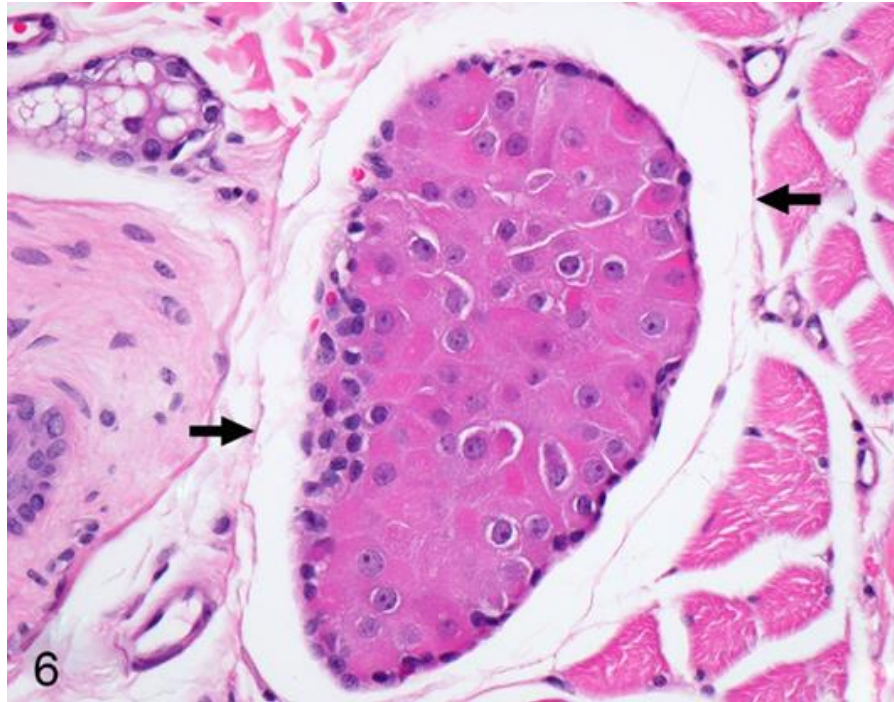


Figure 6: Retraction artifact. Lobule of normal circumanal gland adjacent to a circumanal gland adenoma. Retraction of the connective tissue (arrows) has formed a space around the gland that can be confused with intravascular tumor invasion. The space is not lined by endothelial cells and does not contain blood.

References:

1. Briët JM, Hollema H, Reesink N, et al. Lymphovascular space involvement: an independent prognostic factor in endometrial cancer. *Gynecologic oncology*. 2005;96: 799-804.
2. Falvo L, Catania A, D'Andrea V, Marzullo A, Giustiniani MC, De Antoni E. Prognostic importance of histologic vascular invasion in papillary thyroid carcinoma. *Annals of surgery*. 2005;241: 640.
3. Fitzgibbons PL, Connolly J, Bose S. Protocol for the Examination of Resection Specimens from Patients with Invasive Carcinoma of the Breast. *College of american pathologists*. 2020.
4. Lin X, Zhu B, Liu Y, Silverman JF. Follicular thyroid carcinoma invades venous rather than lymphatic vessels. *Diagnostic pathology*. 2010;5: 1-5.
5. Mete O, Asa SL. Pathological definition and clinical significance of vascular invasion in thyroid carcinomas of follicular epithelial derivation. *Mod Pathol*. 2011;24: 1545-1552.
6. Seethala RR, Asa SL, Carty SE, et al. Protocol for the examination of specimens from patients with carcinomas of the thyroid gland. *College of american pathologists*. 2014.
7. Fujii T, Sutoh T, Morita H, et al. Vascular invasion, but not lymphatic invasion, of the primary tumor is a strong prognostic factor in patients with colorectal cancer. *Anticancer research*. 2014;34: 3147-3151.
8. Fujii T, Yajima R, Hirakata T, et al. Impact of the prognostic value of vascular invasion, but not lymphatic invasion, of the primary tumor in patients with breast cancer. *Anticancer research*. 2014;34: 1255-1259.
9. Peters EE, Bartosch C, McCluggage WG, et al. Reproducibility of lymphovascular space invasion (LVSI) assessment in endometrial cancer. *Histopathology*. 2019;75: 128-136.
10. Pifer P, Bhargava R, Patel A, et al. Is the risk of substantial LVSI in stage I endometrial cancer similar to PORTEC in the North American population?—A single-institution study. *Gynecologic Oncology*. 2020.
11. Goldschmidt M, Pena L, Rasotto R, Zappulli V. Classification and grading of canine mammary tumors. *Vet Pathol*. 2011;48: 117-131.
12. Im KS, Kim NH, Lim HY, Kim HW, Shin JI, Sur JH. Analysis of a new histological and molecular-based classification of canine mammary neoplasia. *Vet Pathol*. 2014;51: 549-559.
13. Mills SW, Musil KM, Davies JL, et al. Prognostic value of histologic grading for feline mammary carcinoma: a retrospective survival analysis. *Vet Pathol*. 2015;52: 238-249.
14. Pena L, De Andres PJ, Clemente M, Cuesta P, Perez-Alenza MD. Prognostic value of histological grading in noninflammatory canine mammary carcinomas in a prospective study with two-year follow-up: relationship with clinical and histological characteristics. *Vet Pathol*. 2013;50: 94-105.
15. Rasotto R, Berlatto D, Goldschmidt MH, Zappulli V. Prognostic Significance of Canine Mammary Tumor Histologic Subtypes: An Observational Cohort Study of 229 Cases. *Vet Pathol*. 2017;54: 571-578.
16. Rasotto R, Zappulli V, Castagnaro M, Goldschmidt MH. A retrospective study of those histopathologic parameters predictive of invasion of the lymphatic system by canine mammary carcinomas. *Vet Pathol*. 2012;49: 330-340.
17. Seixas F, Palmeira C, Pires MA, Bento MJ, Lopes C. Grade is an independent prognostic factor for feline mammary carcinomas: a clinicopathological and survival analysis. *Vet J*. 2011;187: 65-71.
18. Zappulli V, Rasotto R, Caliaro D, et al. Prognostic evaluation of feline mammary carcinomas: a review of the literature. *Vet Pathol*. 2015;52: 46-60.
19. Chocteau F, Abadie J, Loussouarn D, Nguyen F. Proposal for a histological staging system of mammary carcinomas in dogs and cats. Part 1: Canine mammary carcinomas. *Frontiers in Veterinary Science*. 2019;6: 388.
20. Chocteau F, Boulay M-M, Besnard F, Valeau G, Loussouarn D, Nguyen F. Proposal for a histological staging system of mammary carcinomas in dogs and cats. Part 2: Feline mammary carcinomas. *Frontiers in Veterinary Science*. 2019;6.

21. Folkins AK, Nevadunsky NS, Saleemuddin A, et al. Evaluation of vascular space involvement in endometrial adenocarcinomas: laparoscopic vs abdominal hysterectomies. *Mod Pathol*. 2010;23: 1073-1079.
22. Gujam FJ, Going JJ, Mohammed ZM, Orange C, Edwards J, McMillan DC. Immunohistochemical detection improves the prognostic value of lymphatic and blood vessel invasion in primary ductal breast cancer. *BMC Cancer*. 2014;14: 676.
23. Van den Eynden G, Van der Auwera I, Van Laere S, et al. Distinguishing blood and lymph vessel invasion in breast cancer: a prospective immunohistochemical study. *British journal of cancer*. 2006;94: 1643-1649.
24. Zaorsky NG, Patil N, Freedman GM, Tuluc M. Differentiating lymphovascular invasion from retraction artifact on histological specimen of breast carcinoma and their implications on prognosis. *Journal of breast cancer*. 2012;15: 478-480.
25. Nishida T, Katayama S-i, Tsujimoto M. The clinicopathological significance of histologic vascular invasion in differentiated thyroid carcinoma. *The American journal of surgery*. 2002;183: 80-86.
26. Al-Dissi AN, Haines DM, Singh B, Kidney BA. Immunohistochemical expression of vascular endothelial growth factor and vascular endothelial growth factor receptor associated with tumor cell proliferation in canine cutaneous squamous cell carcinomas and trichoepitheliomas. *Vet Pathol*. 2007;44: 823-830.
27. Halsey CH, Worley DR, Curran K, Charles JB, Ehrhart EJ. The use of novel lymphatic endothelial cell-specific immunohistochemical markers to differentiate cutaneous angiosarcomas in dogs. *Vet Comp Oncol*. 2016;14: 236-244.
28. Pusztaszeri MP, Seelentag W, Bosman FT. Immunohistochemical expression of endothelial markers CD31, CD34, von Willebrand factor, and Fli-1 in normal human tissues. *Journal of Histochemistry & Cytochemistry*. 2006;54: 385-395.
29. Sleeckx N, Van Brantegem L, Franssen E, et al. Evaluation of immunohistochemical markers of lymphatic and blood vessels in canine mammary tumours. *J Comp Pathol*. 2013;148: 307-317.
30. Von Beust B, Suter M, Summers B. Factor VIII-related antigen in canine endothelial neoplasms: an immunohistochemical study. *Veterinary Pathology*. 1988;25: 251-255.
31. Wennogle SA, Priestnall SL, Suarez-Bonnet A, Soontararak S, Webb CB. Lymphatic endothelial cell immunohistochemical markers for evaluation of the intestinal lymphatic vasculature in dogs with chronic inflammatory enteropathy. *J Vet Intern Med*. 2019;33: 1669-1676.
32. Ehrensing G, Craig LE. Intravascular neoplastic cells in canine cutaneous plasmacytomas. *J Vet Diagn Invest*. 2018;30: 329-332.
33. El-Gohary YM, Metwally G, Saad RS, Robinson MJ, Mesko T, Poppiti RJ. Prognostic significance of intratumoral and peritumoral lymphatic density and blood vessel density in invasive breast carcinomas. *American journal of clinical pathology*. 2008;129: 578-586.
34. Pak KH, Jo A, Choi HJ, Choi Y, Kim H, Cheong J-H. The different role of intratumoral and peritumoral lymphangiogenesis in gastric cancer progression and prognosis. *BMC cancer*. 2015;15: 498.
35. Zhang S, Zhang D, Gong M, Wen L, Liao C, Zou L. High lymphatic vessel density and presence of lymphovascular invasion both predict poor prognosis in breast cancer. *BMC cancer*. 2017;17: 335.
36. Zhang S, Zhang D, Yi S, et al. The relationship of lymphatic vessel density, lymphovascular invasion, and lymph node metastasis in breast cancer: a systematic review and meta-analysis. *Oncotarget*. 2017;8: 2863.
37. Coomber BL, Denton J, Sylvestre A, Kruth S. Blood vessel density in canine osteosarcoma. *Canadian journal of veterinary research*. 1998;62: 199.
38. Luong R, Baer K, Craft D, Ettinger S, Scase T, Bergman P. Prognostic significance of intratumoral microvessel density in canine soft-tissue sarcomas. *Veterinary pathology*. 2006;43: 622-631.

39. Maiolino P, Papparella S, Restucci B, De Vico G. Angiogenesis in squamous cell carcinomas of canine skin: an immunohistochemical and quantitative analysis. *Journal of Comparative Pathology*. 2001;125: 117-121.
40. Millanta F, Lazzeri G, Vannozi I, Viacava P, Poli A. Correlation of vascular endothelial growth factor expression to overall survival in feline invasive mammary carcinomas. *Veterinary Pathology*. 2002;39: 690-696.
41. Preziosi R, Sarli G, Paltrinieri M. Prognostic value of intratumoral vessel density in cutaneous mast cell tumours of the dog. *Journal of comparative pathology*. 2004;130: 143-151.
42. Restucci B, De Vico G, Maiolino P. Evaluation of angiogenesis in canine mammary tumors by quantitative platelet endothelial cell adhesion molecule immunohistochemistry. *Veterinary Pathology*. 2000;37: 297-301.
43. Restucci B, Maiolino P, Paciello O, Martano M, De Vico G, Papparella S. Evaluation of angiogenesis in canine seminomas by quantitative immunohistochemistry. *Journal of Comparative Pathology*. 2003;128: 252-259.
44. Sleenckx N, Van Brantegem L, Van den Eynden G, et al. Angiogenesis in canine mammary tumours: a morphometric and prognostic study. *Journal of Comparative Pathology*. 2014;150: 175-183.
45. Sleenckx N, Van Brantegem L, Van den Eynden G, et al. Lymphangiogenesis in canine mammary tumours: a morphometric and prognostic study. *Journal of Comparative Pathology*. 2014;150: 184-193.
46. Carroll KA, Kuntz CA, Heller J, Peters A, Rotne R, Dunn A. Tumor size as a predictor of lymphatic invasion in oral melanomas of dogs. *Journal of the American Veterinary Medical Association*. 2020;256: 1123-1128.
47. O'Donnell RK, Feldman M, Mick R, Muschel RJ. Immunohistochemical method identifies lymphovascular invasion in a majority of oral squamous cell carcinomas and discriminates between blood and lymphatic vessel invasion. *J Histochem Cytochem*. 2008;56: 803-810.