Vascular Endothelial Growth Factor in the Aqueous Humor of Dogs With and Without Intraocular Disease

Christina Ann Sandberg

Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

Master of Science
In
Biomedical and Veterinary Sciences

Ian P. Herring, DVM, MS, DACVO
William R. Huckle, MS, PhD
Tanya LeRoith, DVM, PhD, DACVP
John H. Rossmeisl, DVM, MS, DACVIM
J. Phillip Pickett, DVM, DACVO

June 8th, 2009
Blacksburg, VA

Keywords: VEGF, pre-iridal fibrovascular membrane, canine, glaucoma

Vascular Endothelial Growth Factor in the Aqueous Humor of Dogs With and Without Intraocular Disease

Christina A. Sandberg

ABSTRACT

Vascular endothelial growth factor A (VEGF) is a potent mediator of blood vessel formation throughout the body. Intraocular diseases characterized by inflammation, hypoxia or neoplasia induce new blood vessel formation within the eye. The end result of such blood vessel formation may be blinding sequellae such as glaucoma from outflow obstruction or hyphema from intraocular hemorrhage. Elevated VEGF concentrations in the aqueous humor and vitreous are documented in a number of human intraocular disease processes, including tumors, retinal detachment and uveitic glaucoma. Pharmacotherapy inhibiting VEGF expression demonstrates promise for control of some of these ophthalmic conditions. We quantified and compared VEGF concentrations in canine aqueous humor samples from 13 dogs with normal eyes and 226 eyes from 178 dogs with a variety of ophthalmic diseases by ELISA. Dogs with primary cataract, diabetic cataract, primary glaucoma, uveitic glaucoma, aphakic/pseudophakic glaucoma, retinal detachment, lens luxation and neoplasia were evaluated. Elevated VEGF concentrations were found in all disease conditions tested as compared to normal dogs excepting cataracts and diabetic cataracts. Elevated aqueous humor VEGF concentrations were found in dogs with pre-iridal fibrovascular membranes (PIFM) as compared to dogs without PIFM. These results are consistent with the hypothesis that VEGF has a role in the causation or progression of a variety of canine ocular disorders.

ACKNOWLEDGEMENTS

The author recognizes the Virginia-Maryland Regional College of Veterinary Medicine Office of Research and Graduate Studies for providing funding for the project. The author thanks all members of the graduate committee for their assistance throughout the project and their continued support of the author's professional career. In addition, the author greatly appreciates the support of Drs. Phil Pickett, Ian Herring, Jamie Schorling, Daniel Binder, Bill Miller, Albert Mughannam, Stacy Andrew, Heidi Denis, Sandra van der Woerdt, and Patricia Smith in providing clinical samples. The author recognizes Dr. Tanya LeRoith for histopathology review, Dr. Stephen Werre for statistical analyses, Anne Cinsavich and Pamela Arnold for making available normal canine specimens and Betsy Midkiff as the Ophthalmology Technician.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Anatomy and physiology of the canine anterior uvea and iridocorneal angle

The iris and ciliary body comprise the anterior uvea.[1] Embryologically, the iris originates from the neural crest (iris stroma), mesoderm (vascular endothelium) and neuroectoderm (epithelium and muscles).[2] It extends as a diaphragm over the anterior lens and creates the pupil and separates the anterior and posterior chambers of the eye. Histologically, there is an anterior border layer composed of fibroblasts and melanocytes, stroma of collagen, chromatophores and fibroblasts, smooth sphincter and dilator muscles and a posterior epithelial layer.[1,3] Blood supply is via the terminations of the long posterior ciliary arteries, which forms an incomplete arterial circle in domestic animals.[4] Drainage is via the radial vessels to the vortex veins.[5,6] The iris functions to control light passage to the posterior segment and is a highly vascular tissue.[1]

The ciliary body resides posterior to the iris and the posterior iris epithelium is continuous with the ciliary body epithelium. Ciliary body epithelium originates from neuroectoderm and mesenchyme.[2] It is composed of the anterior pars plicata, consisting of approximately 75 ciliary processes in the carnivore, and the posterior pars plana.[1] Smooth muscle comprises the largest mass of this structure. Vascular supply is via the long posterior ciliary arteries to the major arterial circle and anterior ciliary arteries.[4,5] The ciliary body functions to nourish and maintain ocular rigidity by production of aqueous humor, allows accommodation and provides attachment for the lens zonules.[1]

The peripheral anterior iris and ciliary body is bounded by the iridocorneal angle, which is the conventional and major pathway of aqueous humor outflow in the dog.[1,5] During development, the iridocorneal angle develops by separation of the anterior mesenchyme, trabeculae enlargement, trabecular meshwork clefting and postnatal remodeling.[2,7] The pectinate ligament spans the opening of the ciliary cleft. The trabecular meshwork resides within the ciliary cleft and is composed of cell-lined collagenous fibers. Aqueous collecting channels provide outflow from the meshwork and join the angular aqueous plexus to reach systemic circulation via the vortex veins.[1]

Aqueous humor production and outflow pathway in dogs

Aqueous humor is a clear ultra-filtrate of plasma produced by the posterior non-pigmented epithelium of the ciliary processes, primarily by the active transport of solute.[8] It is produced in the posterior chamber, then transverses the pupil to the anterior chamber and exits the eye by the iridocorneal angle.[5] In dogs, the major aqueous outflow pathway is the corneoscleral meshwork; in some other species the uveoscleral or unconventional pathway predominates.[9,10] Balance of aqueous humor production and outflow determines intraocular pressure. The rate of aqueous production may be affected by the health of the ciliary epithelium and intraocular pressure in addition to neural, hormonal and pharmacologic factors. Aqueous outflow may be affected by the health and patency of the drainage pathways, inflammation as well as intraocular pressure.[5,11]

Glaucoma

Glaucoma is a disease typically identified as elevated intraocular pressure caused by obstruction of the normal aqueous humor outflow pathway causing damage to the optic nerve and retinal ganglion cells.[12] Sequellae include pain and permanent blindness; these effects often require surgical removal of the globe or globe contents to alleviate discomfort. Major causes of glaucoma in dogs include inherited or breed-related abnormalities of the iridocorneal angle (known also as primary glaucoma) and outflow obstruction from antecedent intraocular disease (known also as secondary glaucoma).

Primary glaucomas are reported to affect 0.89% of the general canine population, with an increased incidence for specific breeds and lines.[13] Despite anatomical abnormalities of the iridocorneal angle existing throughout life, most dogs with primary glaucoma are affected in middle-age. Inflammation and pigment dispersion in the drainage angle, uvea and retina in the eyes of dogs enucleated for goniodysgenesis-related glaucoma have been reported.[14,15] Tissue ischemia is implicated as cause of retinal injury in dogs.[16]

Dogs with secondary glaucomas have an acquired ocular disease or primary ocular disease unrelated to iridocorneal angle malformation impeding aqueous humor outflow rather than a preexisting structural anomaly of the iridocorneal angle. Secondary glaucomas were diagnosed in 6.9% of dogs presented for evaluation of ophthalmic disease in one veterinary teaching hospital in a 5 year period.[17] The most frequent causes of secondary glaucoma in dogs are anterior uveitis, intraocular surgery, lens dislocation, hyphema, intraocular neoplasia and trauma.[17-19] Additionally, breed-related pigmentary

changes (pigmentary dispersion in the Cairn terrier, uveal cyst syndrome in the Golden Retriever and multiple ciliary body cysts in the Boston Terrier and Great Dane) have been associated with glaucoma.[20-23]

While both medical and surgical anti-glaucoma therapies are widely used for treatment of canine glaucoma, these therapies are rarely effective in controlling the disease long term. Present therapies predominantly reduce aqueous humor production, as exemplified by carbonic anhydrase inhibitors, beta blockers and cyclodestruction, or increase outflow, as exemplified by miotics, prostaglandins and gonioimplants. In the case of secondary glaucomas, therapies are aimed at removing the underlying cause in addition to controlling the resulting intraocular pressure. Lower success rates for control of uveitic glaucomas compared to primary glaucomas are described in dogs, presumably due to secondary effects of ocular inflammation.[12]

Uveitis

In dogs, uveitis or inflammation of the ocular vascular tunic occurs secondary to tissue injury.[24] The blood-aqueous barrier is created by the tight junctions of the nonpigmented ciliary epithelium, the tight junctions and gap junctions of the iris vascular endothelium and the non-fenestrated iridal capillaries.[8] Uveal inflammation induces loss of the normal blood-aqueous barrier via increased vascular permeability and increased vascular supply.

Uveitis has a number of underlying infectious, traumatic, neoplastic, iatrogenic and immune-mediated etiologies. The most commonly diagnosed causes for anterior uveitis in dogs are lens-induced, traumatic, and immune-mediated disease.[19,25,26] Ocular auto-

antigens involved in the initiation of uveitis include lens proteins, and in rare cases, melanocytes.[27,28] Most commonly, an infiltrate of lymphocytes and plasma cells is present in the iris and ciliary body, but the histological findings vary with specific cause.[18]

Sequellae of anterior uveitis include intraocular adhesions (synechiae), cataract, hyphema, secondary glaucoma, and collapse of the globe from chronic hypotony (phthisis bulbi). Mechanisms for uveitic glaucoma include pupil seclusion with annular posterior synechia, angle obstruction with inflammatory debris, peripheral anterior synechia, pre-iridal fibrovascular membrane or a combination of these factors.[24]

Therapies for uveitis include treatment of the underlying cause and non-specific treatment using anti-inflammatory drugs, immunosuppressive agents and immunomodulatory agents. Additionally, specific treatments for prevention or disruption of synechiae and fibrin clots may be employed.[24,26]

Cataract

Cataract is a pathologic opacification of the lens and/or lens capsule and is one of the most common ophthalmic diseases affecting canine patients. Causes include diseases or conditions that alter nutrition, metabolism or osmotic balance of the lens.[18] Cataract formation is common in pet dogs, with higher incidence in some breeds and with increasing age, and cataract extraction surgery is commonly performed by veterinary ophthalmologists.[29,30] Surgical intervention in dogs is indicated for animals with

significant visual impairment from advanced cataract. Most dogs have some degree of intraocular inflammation (phacolytic uveitis) prior to cataract removal.[31-33] It is reported that eyes with clinically significant lens-induced uveitis have a poorer prognosis following cataract extraction.[34] In some cases, cataract formation results in lens capsule rupture and severe intraocular inflammation (phacoclastic uveitis); this condition may be associated with rapid cataract development with or without diabetes mellitus.[35] Complications of cataract surgery include posterior capsular opacification, retinal detachment, postoperative hypertension and glaucoma; an increased risk of long-term glaucoma with hypermature cataract is described.[36]

Intraocular tumors

In dogs, the most common intraocular neoplasms are uveal melanomas, iridociliary epithelial tumors and lymphomas.[18,37,38] Most primary canine uveal tumors arise from the iris or ciliary body and most do not spread beyond the globe.[39,40] However, even benign intraocular tumors may cause extensive damage to delicate structures of the eye, resulting in secondary glaucoma, intraocular hemorrhage and retinal detachment.[24] These sequellae often necessitate enucleation to provide comfort.

Retinal detachment

There are a variety of ophthalmic and systemic conditions contributing to a separation of the neurosensory retina from the retinal pigmented epithelium. Such conditions include retinal tears (rhegmatogenous retinal detachment), chorioretinitis from infectious or autoimmune disease, congenital ocular disease, posterior segment neoplasms, systemic

hypertension or vitreal traction bands.[41] Chronic, extensive retinal detachments result in retinal degeneration, non-responsive uveitis and glaucoma.[42]

Pre-iridal fibrovascular membrane

One key pathologic feature shared by many of the aforementioned diseases is intraocular neovascularization. Iridal neovascularization as described in human beings, consists histologically of a network of blood vessels, with or without myofibroblasts, originating from the superficial iris stroma following antecedent ocular disease. Three stages of neovascularization of the iris in humans are described: new vessels appearing at the iris base and pupillary margin (stage 1), vessels penetrating the anterior iris surface and merging at the collarette (stage 2) and vessels with a connective tissue support (stage 3).[43]

Causes of iris neovascularization in humans are numerous. Broadly, causes include vascular disease (e.g. central retinal vein occlusion), ocular disease (e.g. uveitis, retinal detachment, primary glaucoma and secondary glaucoma), intraocular surgery (e.g. retinal reattachment surgery and cataract extraction), ocular trauma, intraocular neoplasia and systemic disease (e.g. diabetes mellitus).[43] Iridal neovascularization was noted in 0.5% of human eyes removed at autopsy and 18-20% of eyes enucleated for therapeutic purpose.[43]

A well-recognized form of ocular neovascularization that arises secondary to intraocular disease in domestic animals is pre-iridal fibrovascular membrane (PIFM). In domestic

animals, cellular, vascular and fibrous forms of PIFM are described, and are thought to represent a temporal continuum.[44]

Clinically, iridal vascularization may cause reddish discoloration, known as rubeosis iridis; this feature is most apparent in eyes with light colored irides. This discoloration may be absent if the fibroblastic component obscures visualization of vessels.[45] Contracture of the membrane may cause inward or outward turning of the iridal margin, known as entropion or ectropion uveae, respectively. PIFMs may grow across the anterior lens surface to cause pupil seclusion and/or the iridocorneal drainage angle causing obstruction of normal aqueous passage or may extend to the posterior iris or ciliary processes. Due to vessel fragility, PIFMs are prone to rupture and cause intraocular hemorrhage (hyphema). In domestic animals and humans, PIFM formation is suspected to play an important pathophysiologic role in the development of secondary glaucomas.[18,44,46]

PIFMs are routinely noted in dogs with intraocular neoplasms, specifically ciliary body adenoma, adenocarcinoma, neuroepithelial tumor, uveal spindle-cell tumor, and osteosarcoma.[47-51] Additionally, PIFM formation has been identified in dogs with intraocular inflammatory and hypoxic disease, including uveodermatologic syndrome, uveal cyst syndrome, retinal detachment, primary glaucoma, ocular infections and following cataract extraction by phacoemulsification and extracapsular lens extraction.[18,21,23,28,42,52-55]

The incidence of iridal vascularization is widely variable, depending on report and cause. In dogs with retinal detachment, ciliary epithelial neoplasia, chronic glaucoma, ocular hemorrhage, uveal melanoma, endophthalmitis, and uveal cyst syndrome, PIFMs were noted in 21%, 19%, 14%, 10%, 10% and 6% of cases, respectively.[21,44] Histopathologic studies of canine globes enucleated or eviscerated for complications following surgery for cataract removal cite PIFM formation in 86% of cases, with 72% classified as mild, 24% as moderate and 4% as severe.[54] The pathogenesis of PIFM formation in domestic animals has not been elucidated.

Vasculogenesis and Angiogenesis

There are two means of blood vessel growth or development in living systems. Vasculogenesis is the formation of new blood vessels *de novo* and angiogenesis is the development of new blood vessels from pre-existing vasculature. Many factors are implicated in these processes, including vascular endothelial growth factor, basic fibroblast growth factor, platelet derived growth factor, transforming growth factors and angiogenin. Neovascularization by vasculogenesis and/or angiogenesis, plays a key role in growth, tissue repair, inflammation and tumorogenesis.

Vascular endothelial growth factor

Vascular endothelial growth factor (VEGF, referred to also as VEGF-A or vascular permeability factor) is a 46kDa glycoprotein consisting of two 23kDa homodimer proteins, with mitogenic effects on vascular endothelial cell activity causing endothelial cell growth and angiogenesis.[56-58] Both VEGF and its two tyrosine kinase receptors

(VEGFR1/Flt-1 and VEGFR2/KDR or Flk-1) are required for life, as demonstrated by lethality in gene knockout mice models.[59-61]

The *in vivo* roles of VEGF are multi-fold, as this factor has the potential to enhance vascular permeability and to promote new blood vessel formation by aiding the growth and survival of vascular endothelial cells.[62-64] In healthy adult animals, VEGF is expressed in vascularized tissues and theorized to have a role in vascular maintenance by stabilization of mature blood vessels.[65]

There are at least six distinct isoforms of human VEGF, consisting of 121, 145, 165, 183, 189 and 206 amino acids. Alternative exon splicing has a role in phenotypic regulation for both VEGF and VEGF receptors.[66-69] $VEGF_{121}$ and $VEGF_{165}$ are considered more biologically active than other forms. $VEGF_{165}$ is reported to act as more potent pro-inflammatory factor than $VEGF_{121}$.[70] VEGF isoforms are similar across species. Canine VEGF has conserved binding sites and 95.2% and 98.4% sequence homology with human and feline forms, respectively (EMBL accession number AJ133758 for canine VEGF, GenBank accession number AF133250 for canine VEGF and AB071947 for feline VEGF).[69,71,72]

VEGF expression is induced by hypoxia, ischemia, hypo and hyperglycemia, Placental Growth Factor, Transforming Growth Factor-β, Tumor Necrosis Factor-α, and reactive oxygen intermediates.[73-81] Prostaglandin analogs, presumably by induction of hypoxia, also result in elevated VEGF expression.[82] VEGF expression is inhibited by

corticosteroids, steroid hormones and endostatin.[83-86] Reports on the effect of cycloxygenase inhibitors on VEGF expression are conflicting.[84,87,88]

$VEGF_{164}$ is the major isoform expressed in canine tissue.[71] A study in normal dogs employing immunohistochemistry, RT-PCR and real time RT-PCR, revealed strongly positive VEGF expression in Type I and Type II alveolar cells of the lungs, apocrine glands of the skin and corpus leutum. Moderately positive VEGF expression is noted in the Kupffer cells of the liver, lung epithelium, lung smooth muscle, collecting tubule of the kidney, the heart, glomerulosa and reticularis of the adrenal gland, epidermis, hair and sebaceous gland of the skin, and bladder epithelium. Weak VEGF expression is noted in hepatocytes, bile duct, proximal and distal tubule of the kidney, zona fasculata of the adrenal gland, intestinal and bladder muscularis and lymph node. Negative VEGF expression was noted in renal glomerulus, adrenal medulla, thyroid, intestinal epithelium and nerve plexus, pancreas and spleen. The Flt-1 receptor showed similar tissue expression as VEGF. This study did not evaluate expression in ocular tissue.[89]

Within the eye of humans and laboratory animal models, VEGF and/or its receptors are expressed constitutively by numerous tissues apart from vascular endothelium, including the cornea, conjunctiva, iris pigmented epithelium, retinal pigmented epithelium, retinal ganglion cells, astrocytes, Müller cells, and choroidal fibroblasts.[90-93] An autocrine, neuroprotective role of VEGF for Müller cell and photoreceptor survival has been proposed.[94]

In humans, increased ophthalmic VEGF expression is noted in association with a variety of disease conditions. For instance, VEGF over-expression is noted in corneal neovascularization and corneal wound healing.[95,96] Primary open-angle glaucoma, neovascular glaucoma, angle closure glaucoma and exfoliative glaucoma are associated with high levels of VEGF.[97,98] Elevated VEGF expression is present with intraocular tumors, including malignant melanocytes and retinoblastomas.[99-101] In the retina, elevated VEGF is found in central retinal vein occlusion, uveitis, diabetic retinopathy (in retinal tissue and fibrovascular membranes), retinopathy of prematurity, retinitis pigmentosa and age-related macular degeneration.[102-106] In ischemic retinopathies, VEGF expression is noted in iris as well as retinal tissue.[107]

OBJECTIVES

There are numerous canine ophthalmic conditions in which increased VEGF expression may induce intraocular neovascularization and for which control of intraocular neovascularization may limit disease severity or progression. Clarification of the role of VEGF in these conditions may provide rationale for adjunctive therapy, including the use of VEGF inhibitors, in canine patients affected by ocular neovascularization.

The objectives of this study were quantification of aqueous humor VEGF concentrations in dogs affected by a variety of intraocular disease processes and to compare these concentrations with aqueous humor VEGF concentrations in healthy dogs without evidence of ocular disease. Specific ocular disease conditions included primary and diabetic cataracts, primary glaucoma, uveitic glaucoma, intraocular neoplasia, aphakic/pseudophakic glaucoma, retinal detachment, uveitis, lens luxation, ocular trauma, ocular melanosis and uveal cyst disease. Additionally, we sought to compare aqueous humor VEGF levels in eyes with histopathologically confirmed intraocular neovascularization to those without neovascularization.

We hypothesized that aqueous humor VEGF concentrations would correlate with the etiology and chronicity of intraocular disease as well as the presence of intraocular neovascularization.

LITERATURE REVIEW

Role of VEGF in systemic disease

Vascularization mediated by VEGF is implicated in a wide variety of human and canine diseases. These conditions include neoplasia, immune-mediated disease, infectious disease and cardiovascular disease.

Neoplasia

Human beings

In people, VEGF is expressed in patients with carcinoma, sarcoma, adenoma, lymphoma, leukemia and melanoma. For many tumors, VEGF expression can relate to likelihood of disease progression. In a study by Potti *et al*, intratumoral VEGF over-expression was associated with a shorter survival times in patients with soft-tissue sarcoma.[108] Another study by Potti *et al* determined that 20% of archived melanoma samples over-expressed VEGF; however, over-expression of VEGF was not shown to have prognostic value.[109] In head and neck squamous cell carcinomas studied by Eisma *et al,* elevated VEGF expression correlated with aggressive disease, with higher rate of disease recurrence and shorter disease-free interval in patients with tumors expressing higher levels of VEGF.[110] Hui *et al* reported VEGF expression in 60% of nasopharyngeal tumors; high levels of tumor VEGF expression was associated with a poor overall survival.[111] In breast tumors evaluated by Toi *et al*, intratumoral VEGF concentration, especially when soluble VEGF receptor 1 was considered, was a significant poor prognostic indicator.[112] In Jebreel's

study of VEGF expression in thyroid pathologies, elevated VEGF expression was noted in neoplastic disease compared to autoimmune disease.[113]

In some human conditions, circulating blood carries VEGF in elevated quantities, acting as a marker for tumor angiogenesis.[114] Like over-expression within tissue, elevated plasma or serum level of VEGF carries prognostic significance for some diseases. For instance, in patients with hepatocellular carcinoma studied by Poon *et al*, serum level of VEGF higher than 245pg/mL was associated with a shorter overall survival, related to advanced tumor stage and venous invasion.[115] For patients with gastrointestinal cancers evaluated by Hyodo *et a*l, VEGF in plasma greater than 108pg/mL correlated positively with metastatic disease and negatively with response to chemotherapy and survival time.[116] In a study by Etto *et al*, serum VEGF concentrations for patients with non-Hodgkin's lymphoma diminished with treatment from a mean of 500pg/mL at time of diagnosis to 308pg/mL following 6 months of therapy.[117]

Dogs

VEGF levels have also been studied in dogs with neoplastic disease. VEGF expression in canine CNS tumors are reported in three studies. In dogs evaluated by Rossmeisl *et al*, over-expression of VEGF in tumor homogenates by ELISA was found in dogs with intracranial astrocytomas (mean value of 2.84ng/mL, equivalent to 2840pg/mL), oligodendrogliomas (mean value of 0.93ng/mL, equivalent to 930pg/mL) and meningiomas (mean value of 0.24ng/mL, equivalent to 240pg/mL), with a higher expression of VEGF in higher grade tumors.[118] Platt *et al*'s study of dogs with

intracranial meningiomas revealed VEGF expression by immunohistochemistry, with a negative correlation of VEGF expression with survival time.[119] VEGF expression, especially isoform $VEGF_{164}$, was found by Dickinson *et al* using quantitative real-time TaqMan RT-PCR, in a retrospective evaluation of dogs with intracranial tumors; again, tumor grade correlated with VEGF expression.[120]

VEGF expression in canine mammary tumors has also been investigated. In Qui *et al*'s immunohistochemical study of canine mammary tumors, VEGF over-expression was found more often in mammary gland tumors compared to normal tissues and correlated significantly with stage and lymph node metastasis.[121] In a study by Millanta *et al*, clinicopathological variables and prognosis did not correlate with level of VEGF expression by immunohistochemistry in canine mammary carcinomas.[122]

In evaluation of canine cutaneous tumors, Maiolino *et al*'s study found immunohistochemical expression of VEGF in 15/15 of squamous cell carcinomas, but only 3/20 basal cell tumors.[123] In Al-Dissi *et al*'s evaluation of cutaneous tumors by immunohistochemistry, expression of VEGF was found in 89.4% of squamous cell carcinomas and 70.8% of trichepitheliomas and correlated with histological grade for both tumor types.[124] In Patruno *et al*'s evaluation of dogs with spontaneous cutaneous mast cell tumors by ELISA and immunohistochemistry, VEGF expression correlated with microvascular density of tumors and malignancy grade.[125]

VEGF expression has been also evaluated for other canine tumor types. In a study by Wolfsberger *et al* using immunohistochemistry, Western blotting and RT-PCR, VEGF was expressed by 60% of canine lymphomas; VEGFR-1 and VEGFR-2 were expressed by 54% and 7%, respectively, of tumor cells.[126] In Yonomaru *et al*'s study, immunohistochemistry and in-situ hybridization were used to detect VEGF over-expression in vascular tumors, with a higher relative immunoreactivity in hemangiosarcomas compared to hemangiomas, correlating tumor VEGF expression with malignancy.[127] VEGF was detected by immunohistochemistry in canine patients with mastocytoma by Rebuzzi *et al*, but was not believed to act as an autocrine growth regulator.[128]

Circulating and extratumoral VEGF has been evaluated and found to have prognostic implications in a variety of neoplasms in dogs. In a study by Gentili *et al*, serum levels of VEGF by ELISA had prognostic value in lymphoma-affected dogs, positively correlating with stage B disease and negatively correlating with disease-free interval. Mean serum VEGF concentrations for stage A disease were 13.74pg/mL; mean serum VEGF concentration for stage B disease were 38.37pg/mL; mean serum concentrations for healthy control dogs were below the limits of detection.[129] In dogs evaluated by ELISA by Troy *et al*, dogs with neoplastic disease were significantly more likely to have detectable plasma VEGF levels (mean, 17.94pg/mL) compared to healthy dogs (mean, 3.1pg/mL) or dogs with non-neoplastic disease (15/16 dogs below the limits of detection).[130] In dogs with neoplastic and non-neoplastic cavity effusions studied by Clifford *et al* by ELISA, elevated VEGF expression was noted in the effusates compared

to plasma expression. Pericardial and pleural effusates tended to harbor more VEGF than peritoneal effusates, with a median concentration of VEGF of 3533pg/mL, 3144pg/mL and 288pg/mL, respectively.[131]

In dogs with hemangiosarcoma evaluated by Clifford *et al*, a higher proportion of tumor-bearing dogs had detectable plasma VEGF compared to healthy controls (13/16 vs. 1/17). The mean concentration of plasma VEGF in dogs with hemangiosarcoma was 17.2pg/mL.[132] In dogs with mammary tumors studied by Kato *et al*, circulating VEGF levels by ELISA in the plasma and serum were significantly higher than in the healthy control dogs. In healthy dogs, no plasma expression of VEGF was detectable. In diseased dogs, plasma VEGF levels ranged from non detectable to 895.7pg/mL (mean 2.1pg/mL). In healthy dogs, serum concentrations ranged from non detectable to 27.5pg/mL (mean 8.3pg/mL). Mean serum concentrations of dogs with mammary gland tumors ranged from non detectable to 992.9pg/mL (mean 14.85pg/mL). Higher circulating VEGF levels had a positive correlation with malignant tumors and post-operative metastasis.[133] In dogs treated with radiation therapy by Wergin *et al*, baseline plasma VEGF concentrations greater than 5pg/mL were a poor prognostic indicator for outcome. No significant difference between and post radiation treatment plasma VEGF levels were found.[134]

Inflammatory disease

VEGF is also associated with human autoimmune diseases and systemic inflammatory conditions of unknown cause, specifically systemic lupus erythematosus, rheumatoid arthritis, multiple sclerosis and sarcoidosis.[135-137] Serum VEGF levels are reported to correlate with immune reaction type: high levels are noted in autoimmune disease and low levels are noted in allergic reaction[138]

Cardiovascular disease

In a study by Hamada *et al*, inhibitors of VEGF and VEGFR-1 were effective in inhibiting baseline sera activity in patients with cyanotic congenital heart disease.[139] In Hamamichi's study of patients with Kawasaki disease, a syndrome of systemic vasculitis, VEGF expression from neutrophils and mononuclear cells by immunoblot analysis was noted.[140]

In a study by Ray et al of experimental heart failure in dogs, increased VEGF expression was noted by quantitative RT-PCR and was theorized to contribute to altered vasoreactivity leading to pulmonary hypertension.[141]

Anemia

Anemic patients have elevated levels of plasma VEGF by ELISA compared to patients with normal red blood cell count; those with anemia attributed to renal disease had higher VEGF levels than those with malignant disease (16.2pg/mL in normemic patients vs

67.8pg/mL in patients with renal disease vs 49.2pg/mL in patients with malignant disease).[142]

Role of VEGF in Healing

VEGF expression, as detected by immunohistochemistry in a rabbit model, was expressed in epithelial, stromal and endothelial corneal cells during corneal wound healing irrespective of the presence of granulocytes.[96] In Bidder's study of dogs with flexor tendon repair by in situ hybridization, a gradient of VEGF expression was noted in cells at the healing tendon cells, but accumulation within the epitenon was minimal, suggesting a role for VEGF in postoperative angiogenesis.[143] In dogs with experimental distraction osteogenesis studied by Park *et al*, elevated VEGF was noted in Schwann cells and neurovasorum for up to two weeks postoperatively.[144]

VEGF in Ocular disease

In people, increased ophthalmic VEGF expression is noted in association with a variety of disease conditions, including corneal neovascularization, multiple forms of glaucoma, intraocular tumors, uveitis, retinal detachment, central retinal vein occlusion, uveitis, diabetic retinopathy, retinopathy of prematurity, and age-related macular degeneration.[95-106,145-147]

Enzyme-linked immunosorbant assay (ELISA) detection of VEGF from the aqueous humor and vitreous of human beings and animal models has been reported.[97,98,102,148-154] ELISA tests from different manufacturers have variable sensitivity to detection of VEGF.

Intraocular Surgery

Aqueous humor VEGF as assessed by a Biochip Array System (Evidence Investigator, Antrim, N. Ireland) was found by Tu *et al* to be elevated 18 hours following cataract surgery as compared to intra-operative levels (mean values of 463pg/mL vs. 67pg/mL, respectively).[151]

Corneal disease

VEGF has been associated with corneal neovascular disease. Immunohistochemical staining was used by Philipp *et al* to evaluate human corneas with inflammatory disease and normal controls for expression of VEGF and its receptors, Flt-1 and Flk-1. It was found that VEGF expression was increased in vascularized corneas, specially on epithelial cells, vascular endothelial cells and fibroblasts.[95] In a rabbit model by Gan *et al* using immunohistochemistry of corneal alkali burn, VEGF and its Flk-1 receptor were found to be expressed at the leading edge of cornea in epithelial, stromal and endothelial cells during the healing phase; this expression did not require the presence of inflammatory cells.[96]

Intraocular tumors

In studies of human anterior uveal melanomas and melanoma cell lines, expression of high levels of VEGF as assessed by immunohistochemistry, in situ hybridization, Western blot analysis and ELISA have been noted.[101,150,155] In ELISA studies of aqueous humor samples from eyes with uveal melanomas by Missotten *et al* and Boyd *et al*, mean VEGF levels were 146.5pg/mL and 0.8ng (equivalent to 800pg/mL), respectively. Mean

aqueous humor VEGF concentrations of control eyes from these studies were 50.1pg/mL and 0.1ng (equivalent to 100pg/mL), respectively. Missotten et al utilized an ELISA from R&D Systems (Abingdon, Oxford, UK); Boyd et al utilized an ELISA from BioSource International (Camarillo, CA, USA). [150,156]

Glaucoma

Two studies have evaluated the expression of VEGF in the eyes of people with glaucoma. In the first, Tripathi *et al* quantified VEGF expression from the aqueous humor using single antibody competitive binding enzyme immunoassay with an assay range from 0.195 to 200ng/mL (CytImmune Sciences, College Park, MD). In this study, VEGF was detected in all 12 aqueous samples from patients with neovascular glaucoma and yielded a mean VEGF concentration of 29.267ng/mL, equivalent to 29,267pg/mL. VEGF was detected in 15/28 samples from patients with primary open angle glaucoma, with a mean value of 0.726ng/mL, equivalent to 726pg/mL.[97]

In a later study, Hu *et al* quantified aqueous humor VEGF levels in patients with primary, neovascular and uveitic glaucoma using a commercial ELISA kit (R&D Systems, Minneapolis, MN). In this study, VEGF was detected in all samples. Aqueous humor from glaucoma eyes harbored significantly higher VEGF levels than cataracts and there was no significant difference between glaucoma types. In primary open-angle glaucoma samples, there was a mean VEGF concentration of 140.4pg/mL, in angle-closure glaucoma, there was a mean VEGF concentration of 142.8pg/mL and in neovascular glaucoma, there was a mean VEGF concentration of 158.6pg/mL.[98]

In a non-human primate model studied by Tolentino *et al*, 0.25 to 2.5μg VEGF injected intravitreally was sufficient to cause iridal neovascularization and neovascular glaucoma.[157]

Lens disease

In Tripathi *et al's* and Hu *et al's* studies, quantification of VEGF in the aqueous humor of patients with cataracts was also performed. In Tripathi's study, VEGF was detected in 4/20 aqueous humor samples from patients with cataract with a mean concentration of 0.257ng/mL, equivalent to 2570pg/mL. VEGF was detected in 16/16 plasma samples from normal subjects with a mean concentration of 20.246ng/mL, equivalent to 20,246pg/mL.[97] In Hu's study, VEGF was detected in all aqueous humor samples and patients with cataracts had a mean concentration of 102.4pg/mL. VEGF was detected in all plasma samples with a mean concentration of 79.2pg/mL.[98]

Uveitis

In rat and mouse models of autoimmune uveitis by Vinores *et al*, markedly increased retinal expression of VEGF was found by immunohistochemistry. In the same study, moderately increased VEGF expression was noted in retinas with induced ischemic retinopathy.[145] In patients with uveitis evaluated by Fine *et al* using ELISA (R&D Systems, Minneapolis, MN), a correlation with increased aqueous humor VEGF concentrations and cystoid macular edema was made. Mean aqueous humor VEGF concentrations for uveitis patients with and without cystoid macular degeneration were

152 and 109.5pg/mL, respectively.[149] In people with quiescent uveitis studied by Paroli *et al*, elevation in aqueous humor VEGF levels as determined by ELISA (R&D Systems, Abingdon, UK), compared to control subjects was found with values of 118pg/mL and 83pg/mL, respectively.[146]

Retinal disease

Alterations in VEGF have been noted for a variety of retinal conditions, including retinal detachment, diabetic retinopathy and retinitis pigmentosa. In a murine model by Ohno-Matsui *et al*, induction of high levels of VEGF correlated with development of retinal neovascularization, proliferative retinopathy and tractional retinal detachment.[158] Two-fold elevations of retinal VEGF as detected by ELISA (R&D Systems, Minneapolis, MN) were described by Ishida *et al* for rats in a model of pathological neovascularization, compared to rats with physiologic vascular development.[159] In a recent study by Salom *et al*, patients with retinitis pigmentosa, a degenerative retinal disorder, harbored significantly lower levels of VEGF in the aqueous humor as compared to patients with cataracts (mean 94.9pg/mL vs. 336.5pg/mL), as detected by ELISA (Pierce Biotechnology, Woburn, MA).[148]

In patients with retinal detachment, elevated levels of VEGF in the subretinal fluid compared to serum was described by Su *et al* by use of ELISA (R&D Systems, Minneapolis, MN). In cases of simple rhegmatogenous retinal detachment, average VEGF concentration was 355pg/mL, while in cases of proliferative vitreoretinopathy

VEGF level was 901pg/mL; in serum samples, mean VEGF concentration was 168pg/mL.[160]

In a study by Shinoda *et al* using ELISA (R&D Systems, Minneapolis, MN), mean aqueous humor levels of VEGF were significantly higher for patients with proliferative diabetic retinopathy (212 pg/mL) than non-diabetic patients (105pg/mL), patients with non-proliferative retinopathy (77pg/mL) and those without retinopathy (99pg/mL).[102] A study by Ogata *et al* found that patients with diabetic proliferative vitreoretinopathy had significantly higher vitreal levels of VEGF (168μg/mL) than patients with retinal detachment (11μg/mL) using ELISA (manufacturer not specified).[152] Shinoda *et al* found no significant correlation between VEGF levels as detected by ELISA (R&D Systems, Minneapolis, MN) in the aqueous humor in patients with proliferative diabetic retinopathy and the presence of fibrovascular membrane or tractional retinal detachment.[153] Patel *et al* described mean vitreal VEGF concentrations of 957pg/mL in patients with non-proliferative diabetic retinopathy and 596pg/mL in patients with proliferative diabetic retinopathy; patients with proliferative diabetic retinopathy and active neovascularization had higher VEGF levels (1036pg/mL) compared with patients that were in a quiescent phase (303pg/mL).[154]

Adamis *et al* found that intravitreal injection of a VEGF neutralizing antibody inhibited VEGF-mediated capillary endothelial cell proliferation causing iris neovascularization, demonstrating that VEGF is necessary for iridal neovascularization caused by retinal ischemia.[161]

There is a paucity of information concerning VEGF in companion animals with naturally occurring ocular disease. However, one study exists for horses. In horses with spontaneous equine recurrent uveitis studied by Deeg *et al*, focal up-regulation of VEGF was found by Western Blot in the vitreous of affected horses and corresponded to pigment epithelium derived factor down-regulation.[162]

VEGF inhibitors

VEGF inhibitors administered by anterior chamber or intravitreal injection are effective in treatment of some ocular diseases in humans. Intracameral injection of bevacizumab has been shown to decrease aqueous humor VEGF levels in neovascular glaucoma. In a case report by Mason *et al* for three patients with neovascular glaucoma secondary to rubeosis iridis from central retinal vein occlusion, a single intravitreal injection of bevacizumab caused vascular regression and resulted in an intraocular pressure within normal range.[163] Davidorf *et al* described effective use of intravitreal bevacizumab to resolve iridal vascularization in a patient with neovascular glaucoma associated with a choroidal melanoma.[164] Two cases of neovascular glaucoma, and one case of iris/iridocorneal angle neovascularization reported by Chilov *et al*, responded to intravitreal bevacizumab.[165] Wakabayashi *et al* evaluated the outcome of patients in a larger clinical trial with ischemic retinal diseases causing iris neovascularization and neovascular glaucoma and found a favorable outcome for 71% of patients with early disease.[166]

Pegaptanib (trade name Macugen), a pegylated aptamer that blocks $VEGF_{164}$, was the first anti-VEGF therapy approved for ocular use, specifically neovascular (wet) age-related macular degeneration. Selective binding of the drug is theorized to limit side effects from suppression of physiologic VEGF expression. This drug has not shown demonstrable toxicity when administered intravitreally in dogs and rabbits.[167]

Bevacizumab is a recombinant, full-length, anti-VEGF monoclonal antibody. Ranibizumab is a monoclonal antibody fragment, humanized and affinity maturated. As such, ranibizumab has approximately 20 times the binding affinity as bevacizumab and potentially less immunogenicity in humans. *In vitro* testing and testing in animal models have not indicated demonstrable ocular toxicity with intravitreal injection of either drug.[168-170]

Pharmokinetics for these drugs show similarities. In rabbits with 1.25mg of bevacizumab injected intravitreally, drug concentrations in the vitreous declined monoexponentially with a half-life of 4.32 days and aqueous humor drug concentration peaked at 1 week of 29.4ng/mL; concentrations >10ug/mL remained in the vitreous for 30 days.[171] In rabbit models with 0.5mg ranibizumab injected intravitreally, drug concentrations in the vitreous declined monoexponentially with a half life of 2.88 days and aqueous humor drug concentration peaked at 3 days of 17.9ug/mL; concentrations > 0.1ug/mL remained in the vitreous for 29 days.[171]

The long-term safety of these treatments has yet to be evaluated. In cynomolgus monkeys injected with 1.25mg bevacizumab intravitreally, immunoreactivity of the drug was found in blood vessels of the anterior uvea and anterior chamber angle, with the highest levels within 1-4 days following injection.[172] Complications of intravitreal VEGF inhibitor administration are noted, with RPE tears and inflammation reported but considered rare.[173,174]

Bevacizumab is approved in the United States for treatment of metastatic colon cancer under the trade name Avastin (Genentech, Inc., San Francisco, CA). Use of bevacizumab in ophthalmic disease is off-label, as this drug is not approved by the Food and Drug Administration for intraocular use. Currently, ranibizumab (trade name Lucentis) and pegatanib (trade name Macugen), are the two ophthalmic-approved VEGF inhibitors available commercially and labeled for use in neovascular age-related macular degeneration.

A xenogenic VEGF vaccine has been evaluated for use in dogs with soft tissue sarcomas. In a clinical trial of 9 dogs, vaccination over a 16 week period resulted in induction of humoral response to human and canine VEGF; an overall tumor response rate of 30% was noted.[175]

To the best of our knowledge, at this time, VEGF inhibitors have not been evaluated, beyond safety studies, for canine ophthalmic disease.

MATERIALS AND METHODS

Subject Identification

The study population was dogs with intraocular disease presented to the Ophthalmology service at the Virginia-Maryland Regional College of Veterinary Medicine and to participating veterinary ophthalmologists throughout the United States undergoing enucleation, intraocular surgery or requiring aqueous humor sampling for diagnostic or therapeutic purpose. Informed consent was obtained from all clients and all procedures were approved by the Institutional Animal Care and Use Committee of Virginia Polytechnic Institute and State University.

All study subjects had an ophthalmic examination performed by an ACVO board certified veterinary ophthalmologist and/or ophthalmology resident to characterize the ongoing intraocular disease process prior to aqueous humor sampling. Examination entailed slit-lamp biomicroscopy, tonometry and indirect funduscopy or ocular ultrasound.

Ophthalmic or systemic steroid administration and ophthalmic prostaglandin administration within the previous 2 weeks were recorded for each patient. The specific diseases included were cataract, uveitis, intraocular neoplasia, primary glaucoma, post-inflammatory glaucoma, aphakic glaucoma, uveal cyst syndrome and retinal detachment.

The control population consisted of young, adult male mixed breed dogs euthanized at the VTH for reasons unrelated to the present study. There was no apparent adnexal or intraocular disease based on slit-lamp biomicroscopy and indirect funduscopy.

Sample Collection and Handling

Aqueous humor samples were collected by aspiration with a 25 or 27 gauge cannula following corneal incision for intraocular surgery or by perilimbal paracentesis using a 25 or 27 gauge needle. At the time of sampling, we aimed to collect ≥ 0.2mL of aqueous humor. For diseased eyes, aqueous humor was obtained at the time of surgical intervention related to the primary disease process or at the time of aqueous humor collection for other diagnostic or therapeutic purposes. For aqueous humor collection for preliminary assay validation and control dog samples, aqueous humor was collected within 1 hour of euthanasia.

All samples obtained at the Veterinary Teaching Hospital (VTH), were immediately frozen and maintained at -70º C. Samples obtained at distant sites were immediately frozen at -20º C and maintained for up to 6 weeks with overnight transport on ice to the VTH, with subsequent freezing at -70º C. Samples with gross hyphema were centrifuged to remove blood contamination.

A blood sample was obtained from study dogs where possible from peripheral venipuncture or by arterial catheter. Whole blood samples were collected in EDTA-

containing tubes and centrifuged to obtain plasma. Plasma samples were handled and stored identically to aqueous humor samples.

In February, 2008, a freezer malfunction resulted in thawing to room temperature of all previously collected samples. The exact duration of the thaw period was unknown, but may have been up to four days. The thawed samples were replaced to -70^{o} C until time of assay.

VEGF ELISA

A sandwich enzyme-linked immunosorbant assay (ELISA) developed for detection of human VEGF-A in blood, aqueous humor and vitreal samples and previously validated for detection of VEGF in canine blood was utilized for assaying VEGF concentration in canine aqueous humor samples and plasma samples (Quantikine, DVE00, R&D Systems, Minneapolis, MN).[130] Assessment of VEGF concentrations in the aqueous humor and plasma samples was performed in duplicate using 100μL of sample per well according to manufacturer instruction. The mean minimum detectable level of VEGF for this test is reported by the manufacturer to be 9.0 pg/mL and the lowest and highest standards provided are 31.25 pg/mL and 2000pg/mL, respectively. The value of samples below the limits of detection was established to be 0pg/mL.

Sample dilution

In cases where less than 200μL of sample was available, available sample volume was diluted with assay diluent RD6U to a total volume of 100μL and the dilution factor was

noted and used to calculate total VEGF concentration. Samples with greater than 2000pg/mL of VEGF were reassayed after 1:20 or 1:100 dilution with assay diluent RD6U and the dilution factors was noted and used to calculate total VEGF concentration.

Histopathology

Enucleated and eviscerated ocular contents were formalin-fixed and paraffin-embedded. 5μm sections were routinely processed and stained with H&E. For the purposes of the study, a single veterinary pathologist who was unaware of the VEGF level, clinical diagnosis or original histopathologic diagnosis reviewed all slides to evaluate for histopathologic evidence of intraocular neovascularization. PIFMs were classified as cellular, vascular or fibrovascular according to their histopathologic appearance. The lesions were scored as follows: 0 = normal, 1= cellular, 2= fibrous or vascular, 3= fibrovascular.

Aqueous Humor Assay Validation

In order to validate the applicability of the VEGF ELISA assay and confirm its sensitivity and specificity for canine aqueous humor, canine VEGF each was added to pooled canine aqueous humor from dogs without intraocular disease, pooled canine plasma from dogs without systemic disease and assay calibrator diluent RD6U to make 500pg/mL solutions. Solutions were made in triplicate. Assessment of VEGF concentrations in the samples was performed in duplicate using 100μL of sample. The baseline VEGF level of the medium (aqueous humor, plasma or diluent) without added purified canine VEGF was subtracted and the difference was used for subsequent calculations.

Assay of stability in frost-free freezer

The VEGF ELISA test kit manufacturer, R&D systems, recommends freezing samples at temperatures equal to or less than -20º C without repeated freeze-thaw cycles (package insert). The stability of VEGF in standard commercial (frost-free) freezers was evaluated by storing aliquots of pooled aqueous humor samples and plasma samples with purified canine VEGF added to a concentration of 1000pg/mL, in a frost-free freezer for 0, 2, 4, and 6 weeks with subsequent storage at -70^{o}C until assay. Sample preparation and storage was performed in duplicate. Assessment of VEGF concentrations in the samples was performed in duplicate using 100µL of sample.

Assay of stability at room temperature

The VEGF ELISA test kit manufacturer, R&D systems, recommends freezing samples at temperatures equal to or less than -20º C without repeated freeze-thaw cycles (package insert). The stability of VEGF at room temperature for up to 4 days was evaluated by storing aliquots of pooled aqueous humor samples and pooled plasma samples with purified canine VEGF added to a concentration of 1700pg/mL and 500pg/mL at room temperature for 0, 1, 2, 3 or 4 days with subsequent storage at -70º C until assay. Sample preparation and storage was performed in duplicate. Assessment of VEGF concentrations in the samples was performed in duplicate by using 100µL of sample.

Statistical Analysis

Data analyses were performed by a statistician using SAS software (SAS version 9.2, Cary, NC, USA). Aqueous humor VEGF concentrations were log transformed to achieve

normality. Before hypothesis testing, absence of association between plasma VEGF and aqueous humor VEGF (and vice versa) was verified using a scatter plot. Two null hypotheses were stated as: 1) disease condition does not have an effect on aqueous humor VEGF concentration and 2) aqueous humor VEGF concentration does not have an effect on PIFM. To test hypothesis number 1, data were modeled by mixed model ANOVA. Possible confounders comprised categorical (patient sex, breed, systemic disease, glaucoma, concurrent medication, intraocular neovascularization) and continuous (duration and age) variables. In a bivariable model, least squares means (for VEGF concentration) were compared for each of the categorical variables while a regression coefficient was generated for each of the continuous variables. Subsequently each of the hypothesized confounders (one at a time) was added to a multivariable model that included disease and the resulting least squares means (as well as standard errors) for disease compared with those from a model that had only disease as a fixed effect. Where required, the Tukey-Kramer adjustment for multiple comparisons was applied. All models included patient (dog) and sample quality as blocking factors (i.e., random effects). Hypothesis number 2 was tested as described for hypothesis number 1 with PIFM as the main independent variable instead of disease. Significance was set at an α value of 0.05.

RESULTS

Aqueous Humor Assay Validation for 500pg/mL

The mean VEGF concentration of aqueous humor with added standardized canine VEGF was 460pg/mL (95% confidence interval, 433 to 487pg/mL). The mean VEGF concentration of plasma with added standardized canine VEGF was 398pg/mL (95% confidence interval, 371 to 425pg/mL). The mean VEGF concentration of assay diluent RD6U with added standardized canine VEGF were 437pg/mL (95% confidence interval, 410 to 464pg/mL). The concentration of VEGF was significantly greater in aqueous humor than in plasma (p=0.0176). The concentration of VEGF did not differ significantly between aqueous humor and diluent (p=0.3756) or diluent and plasma (p=0.1017).

Assay of stability in frost-free freezer for 1000pg/mL

Aqueous humor samples with added canine VEGF that remained in the -70^{o} freezer at all times between spiking and assay had a mean VEGF concentration of 1020pg/mL (95% confidence interval, 931 to 1110pg/mL); plasma samples with added canine VEGF that remained in the -70^{o} freezer between spiking and assay had a mean VEGF concentration of 980pg/mL (95% confidence interval, 849 to 1110pg/mL). Aqueous humor samples stored in a frost-free -20^{o} freezer for two weeks, four weeks and six weeks after spiking had a mean VEGF concentration of 987pg/mL (95% confidence interval, 899 to 1080pg/mL), 937pg/mL (95% confidence interval, 849 to 1030pg/mL) and 815pg/mL (95% confidence interval, 727 to 903pg/mL), respectively. Plasma samples stored in a -

20^{o} frost-free freezer for two weeks, four weeks and six weeks after spiking had a mean VEGF concentration of 1040pg/mL (95% confidence interval, 909 to 1170pg/mL), 845pg/mL (95% confidence interval, 714 to 975pg/mL) and 917pg/mL (95% confidence interval, 786 to 1050pg/mL), respectively.

The concentration of aqueous humor VEGF for samples continuously stored in -70^{o} freezer and those stored for two weeks in a -20^{o} frost-free freezer were significantly greater than the concentration at week 6 (p=0.0177 and p=0.0471, respectively). The concentration of aqueous humor VEGF for samples continuously stored in -70^{o} freezer did not differ significantly from samples stored in a -20^{o} freezer for two weeks (p=0.9418), or four weeks (p=0.5084). The concentration of plasma VEGF for samples stored in a -20^{o} freezer for two weeks, four weeks and six weeks did not differ significantly from samples maintained at -70^{o} (p=0.8939, p=0.4151 and p=0.8778, respectively).

Assay of VEGF stability in aqueous humor and plasma at room temperature

Samples spiked with canine VEGF to a concentration of 1700pg/mL

Aqueous humor samples with 1700pg/mL canine VEGF that were continuously stored at -70^{o} and those thawed to room temperature for 1 day, 2 days, 3 days and 4 days had a mean VEGF concentration of 1750pg/mL, 1690pg/mL, 1620pg/mL, 1700pg/mL and 1590pg/mL, respectively. Only aqueous humor samples thawed for 4 days showed a significant difference from aqueous humor samples that were continuously stored at -70^{o} (p=0.0227).

Plasma samples with 1700pg/mL canine VEGF that were continuously stored at -70^{o} and those thawed to room temperature for 1 day, 2 days, 3 days and 4 days had a mean VEGF concentration of 1720pg/mL, 1540pg/mL, 1710pg/mL, 1520pg/mL and 1720pg/mL, respectively. Plasma samples with 1700 pg/mL canine VEGF thawed for 4 days did not show a significant difference from plasma samples that were continuously stored at -70^{o} (p=0.9861).

Samples spiked with canine VEGF to a concentration of 500pg/mL

Aqueous humor samples with 500pg/mL of canine VEGF that were continuously stored at -70^{o} and those thawed to room temperature for 1 day, 2 days, 3 days and 4 days had a mean VEGF concentration of 580.2pg/mL, 479.5ppg/mL, 460.3pg/mL, 448.8pg/mL and 427.5pg/mL, respectively. Only aqueous humor samples thawed for 4 days showed a significant decrease from aqueous humor samples that were continuously stored at -70^{o} (p=0.0024).

Plasma samples with 500pg/mL canine VEGF that were continuously stored at -70^{o} and those thawed to room temperature for 1 day, 2 days, 3 days and 4 days had a mean VEGF concentration of 449.2pg/mL, 445.4pg/mL, 426.4pg/mL, 460.3pg/mL and 380.3pg/mL, respectively. Only plasma samples thawed for 4 days showed a significant decrease from aqueous humor and plasma samples that were continuously stored at -70^{o} (p=0.0445).

Descriptive data

All samples

In total, 252 aqueous humor samples and 101 plasma samples from 191 dogs were included in the study. 126 aqueous humor samples were obtained from the right eye, 126 aqueous humor samples were obtained from the left eye; in 30 cases, aqueous humor samples was obtained from both eyes. 43 dogs were mixed breed dogs, 19 were Cocker Spaniels, 16 were Labrador Retrievers, 12 were Jack Russell Terriers, 8 were Beagles, 7 were Bassett Hounds, 6 each were Bichon Frise or Boston Terriers, 5 were Pugs and 69 were other breeds with less than 5 representatives for the breed. Mean age was 6.9 years (range, 9 months to 18 years). 86 had glaucoma and 105 did not have glaucoma. 70 were neutered males, 93 were spayed females, 22 were intact males and 6 were intact females. 140 had no systemic disease, 34 had diabetes mellitus, 4 had cardiac disease, 3 had atopy and 10 had other systemic conditions or disease including pregnancy, urogenital disease, neoplasia, autoimmune disease and infectious disease.

Control

26 aqueous humor samples were obtained from both eyes of 13 dogs without apparent ocular disease and receiving no topical or systemic medications. All control dogs were young adult, intact male, purpose-bred, mixed breed dogs.

Primary cataract

44 aqueous humor samples were obtained from 32 dogs with primary cataract. 21 aqueous humor samples from the right eye, 23 aqueous humor samples from the left eye

and 20 plasma samples were obtained. Breeds of dogs with primary cataract included mixed breed dog (n=4), Bichon Frise (n=3), Boston terrier (n=3), Cocker Spaniel (n=3), Jack Russell Terrier (n=2), others (n=14), Bassett Hound (n=1), Beagle (n=1) and Pug (n=1). Mean age was 6.5 years (range, 1 year to 14 years). 15 dogs were spayed female, 16 were castrated males and 1 was intact female. Mean duration of disease was 4.8 months (range, 1 week to 30 months). 11 dogs received only a topical steroid and 18 dogs received a topical and systemic steroid within 2 weeks of sampling.

Diabetic cataract

51 aqueous humor samples were obtained from 30 dogs with cataract associated with diabetes mellitus. 27 aqueous samples from the right eye, 24 aqueous samples from the left eye and 17 plasma samples were obtained. Breeds of dogs included Labrador Retrievers (n=7), mixed breed dogs (n=8), other (n=10), Bichon Frise (n=2), Pug (n=2) and Beagle (n=1). Mean age was 7.5 years (range, 2 years to 16 years). 15 dogs were spayed female, 13 were castrated males and 2 were intact males. Mean duration of disease was 3.7 months (range, 3 weeks to 18 months). 25 dogs received topical steroids within 2 weeks of sampling.

Lens-induced uveitis

17 aqueous humor samples were obtained from 13 dogs with cataract-related lens-induced uveitis without glaucoma. These dogs were not represented in previous cataract groups. 11 aqueous samples from the right eye, 7 aqueous samples from the left eye and 6 plasma samples were obtained. Breeds represented included other (n=9), Cocker

Spaniel (n=2), Beagle (n=1) and Labrador Retriever (n=1). Mean age was 7.7 years (range, 3 to 13 years). 4 dogs were spayed female, 8 were castrated males, and 1 was an intact male. Mean duration of disease was 1.6 months (range 3 days to 13 months). 12 dogs received topical and 3 dogs received systemic steroids within 2 weeks of sampling.

Aphakic/pseudophakic glaucoma

7 aqueous humor samples were obtained from 7 dogs with aphakic or pseudophakic glaucoma. 4 aqueous samples from the right eye, 3 aqueous samples from the left eye and 6 plasma samples were obtained. Breeds represented included other (n=4), Bichon Frise (n=1), Jack Russell Terrier (n=1) and Cocker Spaniel (n=1). Mean age was 10.1 years (range, 9 years to 12 years). 2 dogs were spayed female and 5 were castrated males. Mean duration of disease was 1.8 months (range, 4 days to 11 months). 6 dogs received topical or systemic steroids within 2 weeks of sampling.

Primary glaucoma

41 aqueous humor samples were obtained from 35 dogs with primary glaucoma. 20 aqueous samples from the right eye, 21 aqueous samples from the left eye and 14 plasma samples were obtained. Breeds of dogs with primary glaucoma included Cocker Spaniels (n=9), mixed breed dogs (n=7), other (n=7), Bassett Hounds (n=6), Jack Russell Terriers (n=3), Labrador Retrievers (n=2) and Pug (n=1). Mean age was 8.6 years (range, 1 year to 14 years). 26 dogs were spayed female, 4 were castrated males, 3 were intact male and 2 were intact female. Mean duration of disease was 2.9 months (range, 3 days to 24

months). 10 dogs received topical or systemic steroids within 2 weeks of sampling and 22 dogs received topical prostaglandins within 2 weeks of sampling.

Uveitic glaucoma

24 aqueous humor samples were obtained from 23 dogs with uveitic glaucoma. 11 aqueous humor samples from the right eye, 13 aqueous humor samples from the left eye and 15 plasma samples were obtained. Breeds represented included other (n=13), mixed breed dogs (n=3), Jack Russell Terrier (n=2), Cocker Spaniels (n=2), Beagle (n=1), Labrador Retriever (n=1) and Pug (n=1). Causes of uveitis included immune-mediated (n=12), trauma (n=3), lens-induced (n=3), corneal perforation (n=2), lymphoma (n=1), and fungal disease (n=1). Mean age was 7.4 years (range, 2 years to 15 years). 11 dogs were spayed females, 11 were castrated male and 1 was an intact female. Mean duration of disease was 0.9 months (range, 1 day to 18 months). 14 dogs received topical steroids, 1 dog received systemic steroids, 1 dog received topical prostaglandin and 3 dogs received topical steroid and prostaglandin within 2 weeks of sampling.

Retinal detachment

13 aqueous humor samples were obtained from 11 dogs with retinal detachments causing glaucoma. 6 plasma samples were obtained. Breeds of dogs with retinal detachments causing glaucoma included Boston Terriers (n=3), Cocker Spaniels (n=2), mixed breed dogs (n=2), others (n=2), Labrador Retriever (n=1), and Beagle (n=1). Mean age was 5.0 years (range, 9 months to 11 years). 4 dogs were spayed female, 5 dogs were castrated males and 2 dogs were intact males. Mean duration of glaucoma was 1.3 months (range,

1 day to 24 months). 2 dogs received topical steroids alone, 3 dogs received topical steroids with topical prostaglandin analogs and 2 dogs received systemic steroids within 2 weeks of sampling.

Intraocular tumor

19 aqueous humor samples were obtained from 19 dogs with intraocular tumors. 9 aqueous samples from the right eye, 10 aqueous samples from the left eye and 12 plasma samples were obtained. Breeds of dogs with intraocular tumors included mixed breed (n=5), Labrador Retrievers (n=4), Beagles (n=2), Jack Russell Terriers (n=1) and others (n=7). Tumor types included benign melanomas (n=8), malignant melanomas (n=4), ciliary body adenomas (n=4), lymphoma (n=1), spindle cell tumor (n=1) and anaplastic tumor (n=1). Mean age was 9.0 years (range, 2 years to 13 years). 13 were spayed females, 5 were castrated males and 1 was intact female. Mean duration of disease was 1.0 months (range, 1 day to 30 months). 10 dogs received topical or systemic steroids within 2 weeks of sampling and 5 dogs received topical prostaglandins within 2 weeks of sampling.

Lens luxation

7 aqueous humor and 3 plasma sample were obtained from 6 dogs with lens luxation causing glaucoma. 3 aqueous samples from the right eye, 4 aqueous samples from the left eye and 3 plasma samples were obtained. Breeds represented included mixed breed (n=1), Jack Russell Terrier (n=1), Beagle (n=1) and other (n=3). Mean age was 7.8 years (range, 3 to 18 years). 3 were spayed females, 1 was an intact female and 3 were

neutered males. Mean duration of disease was 1 week (range, 1 day to 10 months). 3 dogs received topical prostaglandins and 1 dog received topical steroid within 2 weeks of sampling.

Other disease conditions

1 aqueous humor and 1 plasma sample were obtained for 1 dog each with ocular melanosis or uveal cyst syndrome. The dogs represented were a 12 year old male neutered Boxer dog and 11 year old male neutered Golden Retriever, respectively. The duration of disease was 1 week, and 1.5 months, respectively. The dog with uveal cyst syndrome received topical steroids and the dog with ocular melanosis received no medications.

Sample distribution is listed in Table 1.

Aqueous humor VEGF concentration

Control

Vascular endothelial growth factor was detected in 18 of 26 aqueous humor samples from dogs without intraocular disease. Mean concentration of VEGF in aqueous humor samples from control dogs was 10.6pg/mL (95% confidence interval, 2.9 to 39.0pg/mL).

Primary cataract

Vascular endothelial growth factor was detected in 37 of 44 aqueous humor samples from dogs with primary cataract. Mean concentration of VEGF from aqueous humor from dogs with primary cataract was 28.2pg/mL (95% confidence interval, 9.2 to 86.3pg/mL). Mean concentration of VEGF from aqueous humor from 14 eyes of 10 dogs with incomplete cataract was 17.9pg/mL (95% confidence interval, 1.0 to 262pg/mL). Mean concentration of VEGF from aqueous humor from 13 eyes of 9 dogs with complete cataract was 46.1pg/mL (95% confidence interval, 3.3 to 642pg/mL). Mean concentration of VEGF from aqueous humor from 17 eyes of 13 dogs with resorbing cataract was 14.9pg/mL (95% confidence interval, not detectable to 248pg/mL). There was no statistically significant difference in aqueous humor VEGF concentration when comparing cataract stage (incomplete, complete, resorbing) ($p=0.4574$ to 0.9993).

Diabetic cataract

Vascular endothelial growth factor was detected in 43 of 51 aqueous humor samples from dogs with cataract secondary to diabetes mellitus. Mean aqueous VEGF concentration

from dogs with diabetic cataract was 45.0pg/mL (95% confidence interval, 14.7 to 138pg/mL). Mean aqueous VEGF concentration of 9 eyes of 5 dogs with incomplete diabetic cataract was 24.3pg/mL (95% confidence interval, 3.2 to 190pg/mL). Mean aqueous VEGF concentration of 32 eyes of 19 dogs with complete diabetic cataract was 45.6pg/mL (95% confidence interval, 5.3 to 394pg/mL). Mean aqueous VEGF concentration of 10 eyes of 6 dogs with resorbing diabetic cataract was 37.2pg/mL (95% confidence interval, 5.0 to 277pg/mL). There was no statistically significant difference in aqueous humor VEGF concentration when comparing incomplete, complete and resorbing cataract stage (p=0.7125 to 0.9619).

Lens-induced uveitis

Vascular endothelial growth factor was detected in 18 of 18 aqueous humor samples from 13 dogs with cataract-related lens-induced uveitis. Mean aqueous VEGF concentration of dogs with lens-induced uveitis was 664pg/mL (95% confidence interval, 187 to 2360pg/mL). Mean aqueous VEGF concentration of 7 eyes of 4 dogs with complete cataract and lens-induced uveitis was 871pg/mL (95% confidence interval, 266 to 2850pg/mL). Mean aqueous VEGF concentration of 11 eyes of 9 dogs with resorbing cataract and lens-induced uveitis was 708pg/mL (95% confidence interval, 319 to 1570pg/mL). There was no statistically significant difference in aqueous humor VEGF concentration when comparing complete and resorbing cataract stage (p=0.7468).

Primary glaucoma

Vascular endothelial growth factor was detected in 40 of 41 aqueous humor samples from 35 dogs with primary glaucoma. Mean aqueous VEGF concentration of dogs with primary glaucoma was 601pg/mL (95% confidence interval, 197 to 1830pg/mL).

Uveitic glaucoma

Vascular endothelial growth factor was detected in 24 of 24 aqueous humor samples from 23 dogs with glaucoma secondary to uveitis. Mean aqueous VEGF concentration of dogs with uveitic glaucoma was 2150pg/mL (95% confidence interval, 686 to 6710pg/mL).

Aphakic/pseudophakic glaucoma

Vascular endothelial growth factor was detected in 7 of 7 aqueous humor samples from dogs with glaucoma following cataract extraction. Mean aqueous VEGF concentration of 7 dogs with aphakic or pseudophakic glaucoma was 496pg/mL (95% confidence interval, 100 to 2460pg/mL). Mean aqueous VEGF concentration of 5 dogs with pseudophakic glaucoma was 1240pg/mL (95% confidence interval 226 to 6830pg/mL). Concentration of VEGF from aqueous humor of 2 dogs with aphakic glaucoma was 7.8 and 762pg/mL. There was no statistically significant difference in aqueous humor VEGF concentration when comparing aphakia and pseudophakic eyes ($p=0.0799$).

Lens luxation

Vascular endothelial growth factor was detected in 7 of 7 aqueous humor samples from 6 dogs with glaucoma secondary to lens luxation. Mean aqueous VEGF concentration of dogs with lens instability was 1030pg/mL (95% confidence interval, 187 to 5650pg/mL).

Retinal detachment

Vascular endothelial growth factor was detected in 13 of 13 aqueous humor samples from 11 dogs with glaucoma secondary to retinal detachment. Mean aqueous VEGF concentration of dogs with retinal detachment was 3120pg/mL (95% confidence interval, 816 to 11900pg/mL).

Intraocular tumor

Vascular endothelial growth factor was detected in 17 of 19 aqueous humor samples from 19 dogs with intraocular tumors. Aqueous humor VEGF was not detected in 1 patient with benign melanoma and 1 patient with malignant melanoma. Mean aqueous VEGF concentration of dogs with tumors was 1400pg/mL (95% confidence interval, 430 to 4540pg/mL). Mean aqueous VEGF concentration of 8 dogs with benign melanoma was 250pg/mL (95% confidence interval, 12.7 to 4950pg/mL). Mean aqueous VEGF concentration of 4 dogs with malignant melanoma was 1040pg/mL (95% confidence interval, 31.1 to 35000pg/mL). Mean aqueous VEGF concentration of 4 dogs with ciliary body adenoma was 11900pg/mL (95% confidence interval 361 to 38800pg/mL). Concentrations of VEGF from 1 aqueous humor sample each of 1 dog with lymphoma, spindle cell tumor and anaplastic tumor were 2700pg/mL, 3670pg/mL and 63400pg/mL,

respectively. There was not a statistically significant difference when comparing tumor types (p=0.3824 to 1.0).

Other disease conditions

Concentrations of VEGF from the aqueous humor of dogs with ocular melanosis and uveal cyst syndrome were 1250pg/mL, and 815pg/mL, respectively.

Mean aqueous VEGF concentrations are summarized in Table 2 and depicted in Figure 2.

Disease comparison

Aqueous humor VEGF concentration was significantly increased compared to normal dogs in eyes with primary glaucoma ($p<0.001$), aphakic/pseudophakic glaucoma ($p = 0.0018$), lens instability ($p=0.003$), uveitic glaucoma ($p<0.001$), lens-induced uveitis ($p<0.001$), retinal detachment ($p<0.001$) and intraocular tumors ($p<0.0003$). There was no statistically significant difference between dogs without intraocular disease and dogs with primary cataract ($p=0.8237$) or diabetic cataract ($p=0.3419$). There was also no significant difference for aqueous humor VEGF concentration between primary and diabetic cataract ($p=0.9916$).

There was a significant difference for aqueous humor VEGF concentration between primary cataract and all other diseases ($p<0.001$ to 0.0172). There was also a significant difference for aqueous humor VEGF concentration between diabetic cataract and all other diseases ($p<0.01$ to 0.0133). There was not a significant difference between aqueous humor VEGF concentration for any other disease comparison (primary glaucoma, uveitic glaucoma, aphakic/pseudophakic glaucoma, lens-induced uveitis, lens luxation, retinal detachment and intraocular tumor) ($p=0.2652$ to 1.0).

When controlling for disease process, aqueous humor VEGF concentration was not significantly different in diseased eyes of dogs with glaucoma as compared to dogs without glaucoma.

There was no statistically significant difference for aqueous humor VEGF concentration for any breed (p=0.5982 to 1.0) and there was no statistically significant difference of aqueous humor VEGF concentration for any sex excepting intact males as compared to spayed females (p = 0.0332).

There was no statistically significant difference for aqueous humor VEGF concentration in dogs without systemic disease and those with any evaluated systemic disease (e.g. diabetes mellitus, hyperadrenocorticism, cardiac disease, atopy, and hypertension), when analysis was controlled with respect to intraocular disease (p=0.533 to 1.0).

There was no significant difference in aqueous humor VEGF concentration between dogs that received no medication and those that received only topical corticosteroids (p=0.9988). Aqueous humor VEGF concentration in dogs that received topical steroids were significantly lower than dogs that received only topical prostaglandin analogs (p=0.023). Aqueous humor VEGF concentration in dogs that received no medications were significantly higher than in dogs that received topical and systemic steroids (p=0.0002) Aqueous humor VEGF concentration in dogs that received no medications were significantly lower than dogs that received a prostaglandin analog (p=0.0153). There was not a statistically significant difference for aqueous humor VEGF concentration in dogs treated with topical corticosteroids alone as compared to dogs treated with topical and systemic corticosteroids (p=0.1215). Dogs treated with only systemic corticosteroids had a significantly higher aqueous humor VEGF concentration as compared to dogs treated with a topical and systemic steroid (p=0.0263).

When controlling for disease process, aqueous humor VEGF concentration was not significantly correlated with disease duration (data not shown).

There was a tendency for increased aqueous humor VEGF concentration with increasing age. A scatter plot fitted with a regression line gave the following regression equation: $Ln[VEGF]_{AH} = 3.2954 + 0.2413$ x (age), where $[VEGF]_{AH}$ = concentration of VEGF in the aqueous humor in pg/mL and (age) = patient age in years.

Plasma VEGF concentration

Vascular endothelial growth factor was detected in 9 of 21 plasma samples from dogs with primary cataract, with a mean plasma VEGF concentration of 3.2pg/mL (range, 0 to 30.6pg/mL). Vascular endothelial growth factor was detected in 12 of 17 plasma samples from dogs with cataract secondary to diabetes mellitus, with a mean plasma VEGF concentration of 12.0pg/mL (range, 0 to 989pg/mL). Vascular endothelial growth factor was detected in 2 of 6 plasma samples from dogs with lens-induced uveitis with a mean plasma VEGF concentration of 3.7pg/mL (range, 0 to 55.9pg/mL). Vascular endothelial growth factor was detected in 3 of 14 plasma samples from dogs with primary glaucoma, with a mean plasma VEGF concentration of 3.0pg/mL (range, 0 to 46.1pg/mL). Vascular endothelial growth factor was not detected in any of 3 plasma samples from dogs with glaucoma secondary to lens instability or with aphakic glaucoma. Vascular endothelial growth factor was detected in 5 of 15 plasma samples from dogs with uveitic glaucoma, with a mean plasma VEGF concentration of 3.2pg/mL (range, 0 to 44.2pg/mL). Vascular endothelial growth factor was detected in 3 of 6 plasma samples from dogs with retinal detachment, with a mean plasma VEGF concentration of 5.2pg/mL (range, 0 to 33.0pg/mL). Vascular endothelial growth factor was detected in 9 of 12 plasma samples from dogs with intraocular tumor, with a mean plasma VEGF concentration 4.6pg/mL (range, 0 to 23.4pg/mL). Plasma VEGF concentration from 1 dog each with ocular melanosis and uveal cyst syndrome were 27.5pg/mL and 17.9pg/mL, respectively.

Plasma VEGF concentration did not correlate with aqueous humor VEGF concentration. Figure 1 shows an aqueous humor versus plasma VEGF concentration scatter plot with a

fitted regression line for 52 samples in which VEGF was within the limits of detection for both aqueous and plasma samples. A similar scatter plot that included samples where the VEGF concentration was below detection limits and therefore assigned values of zero was evaluated and had a similar regression line, indicating lack of correlation between plasma and aqueous humor VEGF concentrations. Table 4 illustrates the proportions of aqueous and plasma samples that were within and below the detection limits of the assay and their mean values.

Pre-iridal fibrovascular membrane

73 globes were evaluated for pre-iridal fibrovascular membranes (PIFMs). No histopathologic samples were obtained from control dogs or dogs with primary cataract, diabetic cataract or lens-induced uveitis without glaucoma. PIFMs were detected in 56 of 73 (76.7%) histopathologic samples. Of 73 globes examined, 17 (23.3%) had no evidence of a PIFM, 26 (35.6%) had cellular PIFMs, 15 (20.5%) had vascular or fibrous PIFMs and 15 (20.5%) had fibrovascular PIFMs. Of all PIFMs, 46.4% were cellular, 26.8% were vascular or fibrous and 26.8% were fibrovascular.

PIFMs were noted in 10 of 18 (55.6%) histopathologic samples of dogs with primary glaucoma. PIFMs were predominantly cellular (n=7, 70%), with few vascular or fibrous (n=2, 20%) or fibrovascular (n=1, 10%).

PIFMs were noted in 14 of 19 (73.7%) histopathologic samples of dogs with uveitic glaucoma. PIFMs were cellular (n=7, 50%), vascular or fibrous (n=2, 14.3%) and fibrovascular (n=5, 35.7%).

PIFMs were noted in 4 of 4 (100%) histopathologic samples of dogs with glaucoma secondary to lens instability. PIFMs were cellular (n=3, 75%) and vascular (n=1, 25%). PIFMs were noted in 7 of 8 (87.5%) histopathologic samples of dogs with glaucoma secondary to retinal detachment. PIFMs were cellular (n=2, 28.6%), vascular or fibrous (n=2, 28.6%) and fibrovascular (n=3, 42.8%).

PIFMs were noted in 16 of 18 (88.9%) histopathologic samples of dogs with intraocular tumors. In dogs with benign melanomas, PIFMs were predominantly vascular or fibrous (n=4, 57.1%), in addition to cellular (n=1, 14.3%) and fibrovascular (n=2, 28.6%). In dogs with malignant melanoma, PIFMs were vascular or fibrous (n=1, 50%) and fibrovascular (n=1, 50%). In dogs with adenomas, PIFMs were cellular (n=1, 25%), vascular or fibrous (n=2, 50%) and fibrovascular (n=1, 25%).

PIFMs were noted in 1 of 1 histopathologic sample each of dogs with uveal cyst disease and ocular melanosis, both in the cellular form.

PIFM distribution according to disease is listed in Table 3. Examples of cellular, vascular and fibrovascular PIFMs are depicted in figures 3, 4, 5, 6 and 7.

Dogs without evidence of PIFM had a mean aqueous humor VEGF concentration of 417 pg/mL (95% confidence interval, 57.7 to 3020pg/mL). Dogs with cellular PIFM had a mean aqueous humor VEGF concentration of 1061pg/mL (95% confidence interval, 144 to 7840pg/mL). Dogs with vascular or fibrous PIFM had a mean aqueous humor VEGF concentration of 1420pg/mL (95% confidence interval, 196 to 10300pg/mL). Dogs with fibrovascular PIFM had a mean aqueous humor VEGF concentration of 5720pg/mL (95% confidence interval, 791 to 41400pg/mL).

Aqueous humor concentration of VEGF differed significantly for dogs without evidence of PIFM and dogs with a fibrovascular PIFM ($p=0.0012$). Aqueous humor VEGF concentration did not differ significantly for dogs without histopathologic evidence of PIFM as compared to dogs with cellular ($p=0.1704$) and dogs with vascular or fibrous ($p=0.0667$) PIFM. Aqueous humor VEGF concentration did not differ significantly for dogs with cellular PIFM as compared to vascular or fibrous ($p=0.8412$) or fibrovascular ($p=0.1096$) PIFM, nor for dogs with vascular or fibrous as compared to fibrovascular PIFM ($p=0.5255$).

DISCUSSION

Aqueous humor VEGF

In the present study of dogs with intraocular disease, VEGF was detected in aqueous humor in dogs with all disease conditions examined. VEGF expression was below the limits of detection or detected at low levels in plasma and aqueous humor of normal dogs. VEGF concentration in all diseased eyes, excepting primary and diabetic cataracts, was significantly elevated in comparison to non-diseased eyes.

The highest mean aqueous humor VEGF concentrations were found, in descending order, in eyes with retinal detachment, uveitic glaucoma, intraocular tumors, ocular melanosis, lens luxation, uveal cyst syndrome, lens-induced uveitis, primary glaucoma, aphakic/pseudophakic glaucoma. Disease processes as compared to each other were not significantly different, likely due to the broad range and 95% confidence interval of aqueous humor VEGF concentrations found for many disease conditions resulting in overlap between conditions.

The cause of elevated VEGF expression in eyes with these disease conditions may be related, broadly, to the relative induction of ischemia, hypoxia or reactive oxygen intermediates with these diseases. Examination of the exact mechanisms at play is beyond the scope of this study.

Normal dogs

Low levels of VEGF were detected in the aqueous humor of normal dogs. Due to the invasiveness of sampling, aqueous humor VEGF in humans without intraocular disease is not reported and aqueous humor VEGF concentration of other species is not described and so interspecies comparison is not possible. For the purposes of this study, euthanized dogs served as the control population. Despite my best efforts for a timely sampling, in some cases sampling was delayed for up to 1 hour. Post-mortem hypoxia or cellular degradation may be the cause of detectable levels of VEGF in our control samples. While most samples had aqueous humor VEGF concentrations of <20pg/mL, a few samples had levels of 40 to 60pg/mL. A time delay in sample collection may explain the disparity but timing of sample collection was not recorded for each sample. Further study of the post-mortem effects on VEGF in bodily fluids would be required to better elucidate this effect. Additionally, all eyes were evaluated as best possible for pre-existing ocular disease before collection, but histopathology was not performed on any control globes. Some control eyes may have harbored disease below clinical detection limits affecting aqueous humor VEGF concentration.

Cataract

Aqueous humor VEGF concentrations in dogs with primary cataract and diabetic cataract were not significantly elevated compared to normal dogs or compared to each other. Controlled studies in humans often utilize the aqueous humor of patients undergoing cataract extraction for comparison to other diseases, such as glaucoma and intraocular neoplasia. In dogs, intraocular inflammation is noted with all stages of cataract.[31-33] We

found no significant difference in aqueous humor VEGF concentrations in eyes with cataract and eyes without intraocular disease, suggesting either minimal inflammation in these eyes or lack of association of VEGF with subclinical lens-induced uveitis. In humans evaluated with ELISA (R&D Systems, Minneapolis, MN), mean aqueous humor VEGF concentration of 102.4pg/mL was noted, as compared to 28.2pg/mL in dogs noted in the present study.[98]

In relatively few eyes within the cataract groups, aqueous VEGF concentrations reached a very high level. In one patient with primary cataract, aqueous VEGF concentration was 198pg/mL and in one patient with diabetic cataract, aqueous VEGF concentration was 897pg/mL while the majority of patients did not have levels exceeding 100pg/mL. These outliers may represent animals that had subclinical uveitis resulting from the cataract or animals with high levels of oxidative stress at the tissue level.

Subclinical uveitis is reported to be present even in early stages of cataract before clinically apparent uveitis is present.[31-33] Both primary and diabetic cataract samples had lower mean aqueous VEGF concentration as compared to dogs with clinically apparent lens-induced uveitis. The lens is reported to produce inhibitor(s) of angiogenesis.[176] It is possible that this inhibitory effect counteracts the inflammation induced as soluble lens proteins escape the intact lens capsule to produce lens-induced (phacolytic) uveitis. At a point that the regulatory mechanisms are overwhelmed, and vasculogenesis can no longer be staunched, high levels of VEGF may be detected and clinically apparent uveitis may result.

Lens-induced uveitis

Dogs with clinical lens-induced uveitis harbored high aqueous VEGF levels as compared to dogs without disease or with only cataract. Uveitis, or inflammation of the uveal tract, implies a role for inflammatory mediators in order to perpetuate disease. From the results of the present study, VEGF appears to be present in response to intraocular inflammation in dogs. This is similar to what has been described for humans with intraocular inflammation, although lens-induced uveitis is much less common in human patients than dogs and assessment of VEGF expression in this condition for people has not been previously performed, to my best knowledge. Other cytokines are reported to be elevated in human eyes with lens-induced uveitis as compared to chronic uveitis, specifically IFN-γ and IL-12.[177]

Primary glaucoma

Dogs with primary glaucoma had significantly elevated aqueous VEGF concentrations. One major mechanism of injury to the retina with elevated intraocular pressure is ischemia of the retinal ganglion cell layer and optic nerve tissue.[12] This posterior segment hypoxia, as well as ischemic damage to the ocular tissues, provides an explanation for release of a vasogenic factor such as VEGF. Additionally, reactive oxygen intermediates, known to signal VEGF production and release, are involved in the pathogenesis of glaucoma in human beings and likely play a role in dogs.[81,178] Lastly, many dogs with primary glaucomas were treated with prostaglandin analogs. Prostaglandin analogs are reported to induce VEGF expression, although the effects of local administration on aqueous levels have not been reported. There was a statistically

significant increase in aqueous humor VEGF concentration for animals treated with prostaglandin analogs in this study, when controlling data for the disease state.

In a human study utilizing the same kit ELISA (R&D Systems, Minneapolis, MN) as the present study, mean aqueous humor VEGF levels in primary open angle glaucoma patients were 140pg/mL and in angle closure glaucoma were 143pg/mL.[98] Primary glaucomas in dogs are most similar to angle closure glaucoma in humans and the levels we found were 601pg/mL. The 4.3 fold elevation in aqueous humor VEGF in dogs as compared to humans is perhaps related to duration and severity of disease, as most of the canine cases were sampled at the time of salvage surgery, whereas samples from human patients were collected at the time of filtration surgery, presumably earlier in the disease process. Biologic differences in degree of VEGF expression between species may also account for this difference.

Uveitic glaucoma

Uveitic glaucoma shares the ischemia described for primary glaucoma and the inflammation described for lens-induced uveitis. However, dogs with uveitic glaucoma were sampled, in most cases, at the time of a salvage procedure. This implies that these dogs experienced significant, intractable disease. In addition, most dogs were on local and/or systemic medications at the time of sampling. Although these medications were not significantly correlated with aqueous VEGF concentration in this study, they are reported to dampen the production of VEGF.[83,87] It is possible that aqueous VEGF levels

in this study would be more elevated if topical and systemic steroids had not been utilized.

Aqueous humor samples from two people with uveitic glaucoma were evaluated in a study utilizing the same VEGF kit as was utilized in the present study (R&D Systems, Minneapolis, MN). In that report, mean aqueous humor VEGF levels in humans were 322pg/mL, as compared to 2150pg/mL found in dogs with this condition.[98] As was the case for primary glaucomas, the almost 6-fold elevation in aqueous humor VEGF in dogs as compared to humans is perhaps related to duration and severity of disease, as most of the canine cases were sampled at the time of salvage surgery, whereas samples from human patients were collected at the time of filtration surgery, presumably earlier in the disease process. Biologic differences in degree of VEGF expression between species may also contribute to this difference.

Lens instability

The cause of elevated VEGF expression in eyes with lens luxation was most likely related to effects of glaucoma and the inflammation associated with lens instability. In addition, some dogs received topical prostaglandins, which may contribute to elevated aqueous humor VEGF levels, although the contribution of prostaglandins was not found to be statistically significant. There are no human reports, to my best knowledge, which document VEGF or inflammatory cytokine activity in this specific condition in humans, probably due to the uncommon nature of this disease.

Retinal detachment

Retinal detachment in dogs was associated with an elevated aqueous humor VEGF concentration. This finding parallels human disease, where elevated VEGF levels are found in the vitreous and subretinal fluid in patients with retinal detachment.[103,147,160] A specific form of glaucoma in humans with antecedent retinal ischemia is neovascular glaucoma.[43] In a study using the same commercial ELISA utilized for the present study (R&D Systems, Minneapolis, MN), one eye with neovascular glaucoma had an aqueous humor VEGF concentration of 759pg/mL.[98] We found mean aqueous VEGF concentration of 3120pg/mL in dogs with retinal detachment and glaucoma.

Intraocular tumors

In our study, the difference in mean aqueous VEGF concentration between tumor types was not statistically significant. However, it could be expected that differences between tumor types would be related to relative amount of tumor vasculogenesis and host tissue destruction. The cause of elevated VEGF expression in eyes with intraocular tumors may perhaps relate to tumor factors that up regulate VEGF expression in order to mitigate tumor growth or host tissue ischemia following tumor invasion.

VEGF is expressed by human ocular tumors and found in elevated levels in the ocular fluids of eyes with tumor.[99,100,150,155] One study evaluated aqueous humor VEGF concentrations in eyes with uveal melanomas using a similar ELISA kit used for the present study (R&D Systems, Abingdon, Oxford, UK). In that study, aqueous humor VEGF concentrations had a median value of 800pg/mL as compared to dogs in the

present study with mean of 251pg/mL for benign melanoma and 1040pg/mL for malignant melanoma.[156] In this disease process, the malignant melanomas of dogs are more likely to behave biologically like uveal melanomas of humans and VEGF concentrations were indeed similar.

Uveal cyst and ocular melanosis

Pigmentary disease was noted in two patients, one with ocular melanosis and one with uveal cyst syndrome. Both patients had glaucoma. In both conditions, pigment deposits in the eye in varying forms. Since inflammation is not a hallmark for either disease, it is likely that VEGF expression in these conditions is independent of uveitis.[20,21]

In one human patient with exfoliative glaucoma evaluated by the ELISA kit utilized in the present study (R&D Systems, Minneapolis, MN), aqueous humor VEGF concentration of 159pg/mL was detected. In our two patients with pigmentary disease, aqueous humor VEGF concentrations of 1250pg/mL and 815pg/mL were detected. The sample numbers are too low to make meaningful correlations.

Plasma VEGF

Plasma VEGF concentrations were measured to address the possibility that VEGF within the canine eye principally reflects spillover from the systemic circulation. Plasma VEGF exceeded aqueous VEGF in 2 diabetic patients with cataract. In these patients, systemic disease likely played a role in circulating VEGF levels, although systemic disease was not found to be associated with aqueous VEGF concentration when controlling for ocular

disease. For one patient, plasma VEGF was 989pg/mL, significantly higher than the 127 and 135 pg/mL found in the right and left eyes. This is the only patient where circulating VEGF may have influenced aqueous VEGF levels and is unlikely to contribute significantly to the mean of aqueous or plasma VEGF for this group. The other patient had a plasma VEGF concentration of 40pg/mL but aqueous VEGF levels were below the limits of detection. For all other patients, aqueous humor VEGF exceeded plasma VEGF concentration. Aqueous humor VEGF levels in these patients is therefore unlikely to be related to circulating VEGF levels and indicates local VEGF production within the eye.

Our findings of mean plasma VEGF concentrations correlate well with what has been previously described.[118,129,130,132,133] Most dogs had undetectable or low VEGF concentrations in plasma samples. In previous reports, mean plasma utilizing the same ELISA kit (R&D Systems, Minneapolis, MN), plasma VEGF concentrations were largely below the limits of detection for normal dogs.[130] No study has specifically evaluated plasma VEGF in dogs with diabetes mellitus, a condition known to affect circulating VEGF levels in humans.[102] In our study, mean plasma VEGF concentration for dogs with diabetic cataract was 12.0pg/mL.

Pre-iridal fibrovascular membranes

PIFM diagnosis was considerably higher in this study than in previous reports with similar disease conditions. The grading system employed to characterize PIFMs in the present study was based on the descriptions by Peiffer *et al* of PIFM in domestic animals.[44] In our study, PIFMs were detected in 76.7% of ocular samples. The high rate

of PIFM detection presently may be related to increased sensitivity due to high clinical suspicion or an increased severity of disease as compared to previous reports. While case bias may also be implicated, the spectrum of conditions is similar to that examined by Peiffer *et al*.[44]

More specifically, PIFMs were detected in 56% of cases of primary glaucoma in the present study, as compared to 14% of cases of chronic glaucoma by Peiffer *et al*; 88% of cases of retinal detachment in the present study as compared to 21% of globes by Peiffer *et al*; and 89% of cases of intraocular tumor as compared to 19% with ciliary body adenoma and 10% with intraocular melanoma by Peiffer *et al*.[44] However, PIFMs were noted in 86% of globes enucleated for glaucoma following cataract extraction in a previous study, which is comparable to the present report of 75.3% in aphakic or pseudophakic glaucoma.[54] PIFMs were detected in 74% of cases of uveitic glaucoma in this study, a disease condition that has not been evaluated on a large scale previously in the dog.

The cellular, vascular and fibrous forms of PIFM are theorized to represent a spectrum of progression with chronicity of disease.[44] Of all PIFMs detected in the present study, the cellular form predominated. The fibrovascular form was significantly associated with elevated VEGF concentration as compared to eyes that did not have evidence of PIFM, which may indicate that aqueous humor VEGF is responsible for the vascular component of PIFM or that the PIFM is responsible for VEGF expression. The cellular and vascular forms of PIFM did not vary significantly from the eyes without PIFM or the eyes with

fibrovascular PIFM. It would be most advantageous to correlate PIFM with VEGF and/or VEGF receptor expression to better confirm a causative relationship of VEGF on PIFM development.

Primary glaucoma

Dogs with primary glaucoma tended to have no evidence of a PIFM (8/18) or have a cellular PIFM (7/18) on histopathology. Only 1 dog of 18 had a fibrovascular PIFM, the only PIFM type which correlated with elevated aqueous humor VEGF levels. Although cellular to fibrovascular forms of PIFMs are thought to represent a continuum, the finding that many dogs in this group, despite end-stage disease, did not develop a PIFM or show evidence of progression past a cellular form may indicate that ocular neovascularization is not an overwhelming response to primary glaucoma in dogs.

Uveitic glaucoma

In dogs with uveitic glaucoma studied here, 14/19 eyes had evidence of a PIFM in a cellular (7/19) to fibrovascular (5/19) form. The high aqueous humor VEGF concentration in concert with the high degree of intraocular neovascularization supports a pathogenic role for VEGF and PIFM in canine uveitic glaucomas.

Lens luxation

In the present study, 4 of 4 dogs with lens luxation had PIFM, 3 in a cellular and 1 in a vascular form. This demonstrates that few of these dogs have the more progressed fibrovascular form and parallels the findings for primary glaucoma.

Retinal detachment

Dogs with retinal detachment had evidence of PIFM in 7 of 8 histopathology samples. Fibrovascular PIFM were found in 37.5% of retinal detachment cases, the highest relative frequency of any disease process we evaluated. The high mean VEGF concentration and high frequency of fibrovascular PIFM gives strong indication for a causal association between these two variables in dogs with retinal detachment. These findings explain the incidence of hyphema and secondary glaucoma in dogs with retinal detachment.[41]

Intraocular tumors

Overall, dogs with intraocular tumors had a fairly high rate of PIFM. Of 18 dogs, 6/18 dogs had fibrovascular PIFM, and 7/18 had vascular PIFM. Only 2 dogs did not have evidence of PIFM. Analyzing specific groups, for cases of benign melanomas, 2/8 had fibrovascular PIFM and 4/8 had vascular PIFM. For malignant melanoma, 1/3 had fibrovascular and 1/3 had vascular PIFM. For adenoma, 1/4 had fibrovascular and 2/4 had vascular PIFM. Overall, there was a high incidence of fibrovascular and vascular PIFM as compared to cellular PIFM, again supporting a pathogenic role for VEGF in the development of complications, particularly secondary glaucoma, from intraocular neoplasms.

Uveal cyst and ocular melanosis

Dogs with melanosis and uveal cyst syndrome each had cellular form of PIFM.

Caveats

Limitations of this study include sampling and patient factors. A limited number of samples were obtained from some dogs with specific disease conditions, notably uveal cyst syndrome and ocular melanosis, rendering it impossible to draw concrete conclusions from these disease groups.

Within disease groups, subgroups of the disease conditions had limited case numbers, especially tumors and lens regrowth. Increasing the number of dogs with specific conditions may have allowed elucidation of differences within groups.

There was a wide range of VEGF levels for many disease conditions. This allowed a large amount of overlap between disease groups and often prevented conclusions regarding significant differences between groups to be drawn.

As a prospective clinical study, there is an aim for consistency. However, as an uncontrolled, non-randomized study of clinical patients, control of some factors is not practical or possible. In this study we reported, but did not control, medications utilized, patient signalment, duration of disease and systemic disease.

There was a sincere goal to treat all patient samples identically from the time of collection until assay, but this aim was thwarted by failed refrigeration midway through the project, thawing all samples collected to that point to room temperature for up to 4 days. We attempted to replicate this room temperature storage for samples spiked with a

controlled amount of VEGF. However, these experiments revealed a small, but statistically significant, decline in sample VEGF, significant for the fourth day. A decline in aqueous humor VEGF concentration over time would not render this study invalid as it would not likely change our findings in a clinically significant manner.

CONCLUSIONS

The purpose of this study was to evaluate expression of vascular endothelial growth factor in the aqueous humor of dogs with intraocular disease.

An ELISA kit targeting human $VEGF_{164}$ was used successfully to detect VEGF in the aqueous humor of normal dogs and the aqueous humor and plasma of dogs with a variety of intraocular diseases.

Based on the results of this study, VEGF is expressed at low levels in dogs without intraocular disease and in dogs with cataract. VEGF expression is significantly elevated in the aqueous humor of dogs with inflammatory and hypoxic intraocular diseases, specifically retinal detachment, uveitic glaucoma, intraocular tumors, ocular melanosis, lens luxation, uveal cyst syndrome, lens-induced uveitis, primary glaucoma, aphakic/pseudophakic glaucoma. Whether VEGF levels are merely elevated in response to disease or play a pathogenic role in these conditions likely varies with disease and remains to be elucidated.

Aqueous humor VEGF concentration is positively correlated with detection of the fibrovascular form of pre-iridal fibrovascular membrane and likely has a pathogenic role in that condition.

VEGF appears to be produced within the canine eye in response to disease. The tissues responsible for such production remain to be elucidated.

These results indicate a role for VEGF inhibitors early in the treatment of dogs with inflammatory and hypoxic ocular disease in order to prevent PIFM formation. It is warranted to continue research regarding the safety and efficacy of such compounds for use in companion animals, specifically dogs, as these medications may limit the sequellae of diseases characterized by intraocular neovascularization, most specifically retinal detachment, uveal tumors and uveitic glaucoma.

LITERATURE CITED

References

1. Samuelson D. Ophthalmic anatomy In: Gelatt KN, ed. *Veterinary Ophthalmology*. 4th ed. Ames, IA: Blackwell Publishing, 2007;62-88.
2. Cook C. Ocular Embryology and Congenital Malformations In: Gelatt KN, ed. *Veterinary Ophthalmology*. 4th ed. Ames, IA: Blackwell Publishing, 2007;14-16.
3. Donovan RH, Carpenter RL, Schepens CL, et al. Histology of the normal collie eye II. Uvea. *Ann Ophthalmol* 1974;6:1175-1178, 1181-1172, 1185-1176 passim.
4. Sharpnack DD, Wyman M, Anderson BG, et al. Vascular pathways of the anterior segment of the canine eye. *American Journal of Veterinary Research* 1984;45:1287-1294.
5. Gum GG GK, Esson DW. Physiology of the Eye In: Gelatt KN, ed. *Veterinary Ophthalmology*. 4th ed. Ames, IA: Blackwood Publishing, 2007;158-170.
6. Anderson BG, Anderson WD. Vasculature of the equine and canine iris. *Am J Vet Res* 1977;38:1791-1799.
7. Samuelson DA, Gelatt KN. Aqueous outflow in the beagle. I. Postnatal morphologic development of the iridocorneal angle: pectinate ligament and uveal trabecular meshwork. *Curr Eye Res* 1984;3:783-794.
8. Caprioli J. The Ciliary Epithelia and Aqueous Humor In: WM H, ed. *Adler's Physiology of the Eye*. 9th ed. St Louis, MO: Mosby-Year Book, 1992;228-243.
9. Samuelson DA, Gum GG, Gelatt KN, et al. Aqueous outflow in the beagle: unconventional outflow, using different-sized microspheres. *Am J Vet Res* 1985;46:242-248.
10. Smith PJ, Samuelson DA, Brooks DE, et al. Unconventional aqueous humor outflow of microspheres perfused into the equine eye. *Am J Vet Res* 1986;47:2445-2453.
11. Toris CB, Pederson JE. Aqueous humor dynamics in experimental iridocyclitis. *Invest Ophthalmol Vis Sci* 1987;28:477-481.
12. Gelatt KN BD, Kallberg ME. The Canine Glaucomas In: KN G, ed. *Veterinary Ophthalmology*. 4th ed. Ames, IA: Blackwell Publishing, 2007;753-802.
13. Gelatt KN, MacKay EO. Prevalence of the breed-related glaucomas in pure-bred dogs in North America. *Vet Ophthalmol* 2004;7:97-111.
14. Reilly CM, Morris R, Dubielzig RR. Canine goniodysgenesis-related glaucoma: a morphologic review of 100 cases looking at inflammation and pigment dispersion. *Vet Ophthalmol* 2005;8:253-258.
15. Mangan BG, Al-Yahya K, Chen CT, et al. Retinal pigment epithelial damage, breakdown of the blood-retinal barrier, and retinal inflammation in dogs with primary glaucoma. *Vet Ophthalmol* 2007;10 Suppl 1:117-124.
16. Savagian CA, Dubielzig RR, Nork TM. Comparison of the distribution of glial fibrillary acidic protein, heat shock protein 60, and hypoxia-inducible factor-1alpha in retinas from glaucomatous and normal canine eyes. *Am J Vet Res* 2008;69:265-272.
17. Johnsen DA, Maggs DJ, Kass PH. Evaluation of risk factors for development of secondary glaucoma in dogs: 156 cases (1999-2004). *J Am Vet Med Assoc* 2006;229:1270-1274.

18. Grahn BH PR. Fundamentals of Veterinary Ophthalmic Pathology In: Gelatt KN, ed. *Veterinary Ophthalmology*. 4th ed. Ames, IA: Blackwood Publishing, 2007;402-408.
19. Gelatt KN, MacKay EO. Secondary glaucomas in the dog in North America. *Vet Ophthalmol* 2004;7:245-259.
20. Petersen-Jones SM, Forcier J, Mentzer AL. Ocular melanosis in the Cairn Terrier: clinical description and investigation of mode of inheritance. *Vet Ophthalmol* 2007;10 Suppl 1:63-69.
21. Deehr AJ, Dubielzig RR. A histopathological study of iridociliary cysts and glaucoma in Golden Retrievers. *Vet Ophthalmol* 1998;1:153-158.
22. Corcoran KA, Koch SA. Uveal cysts in dogs: 28 cases (1989-1991). *J Am Vet Med Assoc* 1993;203:545-546.
23. Spiess BM, Bolliger JO, Guscetti F, et al. Multiple ciliary body cysts and secondary glaucoma in the Great Dane: a report of nine cases. *Vet Ophthalmol* 1998;1:41-45.
24. Hendrix D. Diseases and Surgery of the Canine Anterior Uvea In: Gelatt KN, ed. *2007*. 4th ed. Ames, IA: Blackwell Publishing, 2007;812-849.
25. Massa KL, Gilger BC, Miller TL, et al. Causes of uveitis in dogs: 102 cases (1989-2000). *Vet Ophthalmol* 2002;5:93-98.
26. Townsend WM. Canine and feline uveitis. *Vet Clin North Am Small Anim Pract* 2008;38:323-346, vii.
27. Wilcock BP, Peiffer RL, Jr. The pathology of lens-induced uveitis in dogs. *Vet Pathol* 1987;24:549-553.
28. Sigle KJ, McLellan GJ, Haynes JS, et al. Unilateral uveitis in a dog with uveodermatologic syndrome. *J Am Vet Med Assoc* 2006;228:543-548.
29. Gelatt KN, Mackay EO. Prevalence of primary breed-related cataracts in the dog in North America. *Vet Ophthalmol* 2005;8:101-111.
30. Wilkie DA, Colitz CM. Update on veterinary cataract surgery. *Curr Opin Ophthalmol* 2009;20:61-68.
31. Paulsen ME, Lavach JD, Severin GA, et al. The effect of lens-induced uveitis on the success of extracapsular cataract extraction: a retrospective study of 65 lens removals in the dog. *Journal of the American Animal Hospital Association* 1986;22:49-56.
32. Dziezyc J, Millichamp NJ, Smith WB. Fluorescein concentrations in the aqueous of dogs with cataracts. *Veterinary & Comparative Ophthalmology* 1997;7:267-270.
33. Leasure J, Gelatt KN, MacKay EO. The relationship of cataract maturity to intraocular pressure in dogs. *Vet Ophthalmol* 2001;4:273-276.
34. van der Woerdt A, Nasisse MP, Davidson MG. Lens-induced uveitis in dogs: 151 cases (1985-1990). *J Am Vet Med Assoc* 1992;201:921-926.
35. Wilkie DA, Gemensky-Metzler AJ, Colitz CM, et al. Canine cataracts, diabetes mellitus and spontaneous lens capsule rupture: a retrospective study of 18 dogs. *Vet Ophthalmol* 2006;9:328-334.
36. Sigle KJ, Nasisse MP. Long-term complications after phacoemulsification for cataract removal in dogs: 172 cases (1995-2002). *J Am Vet Med Assoc* 2006;228:74-79.
37. Dubielzig RR. Ocular neoplasia in small animals. *Vet Clin North Am Small Anim Pract* 1990;20:837-848.

38. Krohne SG, Henderson NM, Richardson RC, et al. Prevalence of ocular involvement in dogs with multicentric lymphoma: prospective evaluation of 94 cases. *Veterinary & Comparative Ophthalmology* 1994;4:127-135.
39. Wilcock BP, Peiffer RL, Jr. Morphology and behavior of primary ocular melanomas in 91 dogs. *Vet Pathol* 1986;23:418-424.
40. Ryan AM, Diters RW. Clinical and pathologic features of canine ocular melanomas. *J Am Vet Med Assoc* 1984;184:60-67.
41. Narfstrom K PJS. Diseases of the Canine Ocular Fundus In: KN G, ed. *Veterinary Ophthalmology*. 4th ed. Ames, IA: Blackwell Publishing, 2007;1007-1012.
42. Grahn BH, Barnes LD, Breaux CB, et al. Chronic retinal detachment and giant retinal tears in 34 dogs: outcome comparison of no treatment, topical medical therapy, and retinal reattachment after vitrectomy. *Can Vet J* 2007;48:1031-1039.
43. Gartner S, Henkind P. Neovascularization of the iris (rubeosis iridis). *Surv Ophthalmol* 1978;22:291-312.
44. Peiffer RL, Jr., Wilcock BP, Yin H. The pathogenesis and significance of pre-iridal fibrovascular membrane in domestic animals. *Vet Pathol* 1990;27:41-45.
45. John T, Sassani JW, Eagle RC, Jr. The myofibroblastic component of rubeosis iridis. *Ophthalmology* 1983;90:721-728.
46. Barile GR, Chang S, Horowitz JD, et al. Neovascular complications associated with rubeosis iridis and peripheral retinal detachment after retinal detachment surgery. *Am J Ophthalmol* 1998;126:379-389.
47. Dubielzig RR, Steinberg H, Garvin H, et al. Iridociliary epithelial tumors in 100 dogs and 17 cats: a morphological study. *Vet Ophthalmol* 1998;1:223-231.
48. Peiffer RL, Jr. Ciliary body epithelial tumours in the dog and cat; a report of thirteen cases. *Journal of Small Animal Practice* 1983;24:347-370.
49. Hendrix DV, Donnell RL. Lenticular invasion by a ciliary body adenocarcinoma in a dog. *Vet Pathol* 2007;44:540-542.
50. Aleksandersen M, Bjerkas E, Heiene R, et al. Malignant teratoid medulloepithelioma with brain and kidney involvement in a dog. *Vet Ophthalmol* 2004;7:407-411.
51. Heath S, Rankin AJ, Dubielzig RR. Primary ocular osteosarcoma in a dog. *Vet Ophthalmol* 2003;6:85-87.
52. Sapienza JS, Simo FJ, Prades-Sapienza A. Golden Retriever uveitis: 75 cases (1994-1999). *Vet Ophthalmol* 2000;3:241-246.
53. Zarfoss MK, Dubielzig RR, Eberhard ML, et al. Canine ocular onchocerciasis in the United States: two new cases and a review of the literature. *Vet Ophthalmol* 2005;8:51-57.
54. Moore DL, McLellan GJ, Dubielzig RR. A study of the morphology of canine eyes enucleated or eviscerated due to complications following phacoemulsification. *Vet Ophthalmol* 2003;6:219-226.
55. Collinson PN, Peiffer RL, Jr. Pathology of canine cataract surgery complications. *N Z Vet J* 2002;50:26-31.
56. Gospodarowicz D, Abraham JA, Schilling J. Isolation and characterization of a vascular endothelial cell mitogen produced by pituitary-derived folliculo stellate cells. *Proc Natl Acad Sci U S A* 1989;86:7311-7315.

57. Ferrara N, Henzel WJ. Pituitary follicular cells secrete a novel heparin-binding growth factor specific for vascular endothelial cells. *Biochem Biophys Res Commun* 1989;161:851-858.
58. Leung DW, Cachianes G, Kuang WJ, et al. Vascular endothelial growth factor is a secreted angiogenic mitogen. *Science* 1989;246:1306-1309.
59. Ferrara N, Carver-Moore K, Chen H, et al. Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene. *Nature* 1996;380:439-442.
60. Fong GH, Rossant J, Gertsenstein M, et al. Role of the Flt-1 receptor tyrosine kinase in regulating the assembly of vascular endothelium. *Nature* 1995;376:66-70.
61. Shalaby F, Rossant J, Yamaguchi TP, et al. Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice. *Nature* 1995;376:62-66.
62. Ferrara N, Davis-Smyth T. The biology of vascular endothelial growth factor. *Endocr Rev* 1997;18:4-25.
63. Alon T, Hemo I, Itin A, et al. Vascular endothelial growth factor acts as a survival factor for newly formed retinal vessels and has implications for retinopathy of prematurity. *Nat Med* 1995;1:1024-1028.
64. Murata T, Ishibashi T, Khalil A, et al. Vascular endothelial growth factor plays a role in hyperpermeability of diabetic retinal vessels. *Ophthalmic Res* 1995;27:48-52.
65. Maharaj AS, Saint-Geniez M, Maldonado AE, et al. Vascular endothelial growth factor localization in the adult. *Am J Pathol* 2006;168:639-648.
66. Tischer E, Mitchell R, Hartman T, et al. The human gene for vascular endothelial growth factor. Multiple protein forms are encoded through alternative exon splicing. *J Biol Chem* 1991;266:11947-11954.
67. Woolard J, Wang WY, Bevan HS, et al. VEGF165b, an inhibitory vascular endothelial growth factor splice variant: mechanism of action, in vivo effect on angiogenesis and endogenous protein expression. *Cancer Res* 2004;64:7822-7835.
68. Houck KA, Ferrara N, Winer J, et al. The vascular endothelial growth factor family: identification of a fourth molecular species and characterization of alternative splicing of RNA. *Mol Endocrinol* 1991;5:1806-1814.
69. Scheidegger P, Weiglhofer W, Suarez S, et al. Vascular endothelial growth factor (VEGF) and its receptors in tumor-bearing dogs. *Biol Chem* 1999;380:1449-1454.
70. Usui T, Ishida S, Yamashiro K, et al. VEGF164(165) as the pathological isoform: differential leukocyte and endothelial responses through VEGFR1 and VEGFR2. *Invest Ophthalmol Vis Sci* 2004;45:368-374.
71. Jingjing L, Srinivasan B, Bian X, et al. Vascular endothelial growth factor is increased following coronary artery occlusion in the dog heart. *Mol Cell Biochem* 2000;214:23-30.
72. Koga L, Kobayashi Y, Yazawa M, et al. Nucleotide sequence and expression of the feline vascular endothelial growth factor. *J Vet Med Sci* 2002;64:453-456.
73. Shweiki D, Itin A, Soffer D, et al. Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. *Nature* 1992;359:843-845.
74. Vasir B, Aiello LP, Yoon KH, et al. Hypoxia induces vascular endothelial growth factor gene and protein expression in cultured rat islet cells. *Diabetes* 1998;47:1894-1903.

75. Matsunaga T, Warltier DC, Weihrauch DW, et al. Ischemia-induced coronary collateral growth is dependent on vascular endothelial growth factor and nitric oxide. *Circulation* 2000;102:3098-3103.
76. Stein I, Neeman M, Shweiki D, et al. Stabilization of vascular endothelial growth factor mRNA by hypoxia and hypoglycemia and co-regulation with other ischemia-induced genes. *Mol Cell Biol* 1995;15:5363-5368.
77. Mu H, Zhang XM, Liu JJ, et al. Effect of high glucose concentration on VEGF and PEDF expression in cultured retinal Muller cells. *Mol Biol Rep* 2008.
78. Bottomley MJ, Webb NJ, Watson CJ, et al. Placenta growth factor (PlGF) induces vascular endothelial growth factor (VEGF) secretion from mononuclear cells and is co-expressed with VEGF in synovial fluid. *Clin Exp Immunol* 2000;119:182-188.
79. Pertovaara L, Kaipainen A, Mustonen T, et al. Vascular endothelial growth factor is induced in response to transforming growth factor-beta in fibroblastic and epithelial cells. *J Biol Chem* 1994;269:6271-6274.
80. Ryuto M, Ono M, Izumi H, et al. Induction of vascular endothelial growth factor by tumor necrosis factor alpha in human glioma cells. Possible roles of SP-1. *J Biol Chem* 1996;271:28220-28228.
81. Kuroki M, Voest EE, Amano S, et al. Reactive oxygen intermediates increase vascular endothelial growth factor expression in vitro and in vivo. *J Clin Invest* 1996;98:1667-1675.
82. Girsh E, Plaks V, Gilad AA, et al. Cloprostenol, a prostaglandin F(2alpha) analog, induces hypoxia in rat placenta: BOLD contrast MRI. *NMR Biomed* 2007;20:28-39.
83. Nauck M, Karakiulakis G, Perruchoud AP, et al. Corticosteroids inhibit the expression of the vascular endothelial growth factor gene in human vascular smooth muscle cells. *Eur J Pharmacol* 1998;341:309-315.
84. Edelman JL, Lutz D, Castro MR. Corticosteroids inhibit VEGF-induced vascular leakage in a rabbit model of blood-retinal and blood-aqueous barrier breakdown. *Exp Eye Res* 2005;80:249-258.
85. Sibug RM, Helmerhorst FM, Tijssen AM, et al. Estrogen reduces vascular endothelial growth factor(164) expression in the mouse nucleus paraventricularis of the hypothalamus. *Neurosci Lett* 2002;333:199-202.
86. Takahashi K, Saishin Y, Saishin Y, et al. Intraocular expression of endostatin reduces VEGF-induced retinal vascular permeability, neovascularization, and retinal detachment. *Faseb J* 2003;17:896-898.
87. Castro MR, Lutz D, Edelman JL. Effect of COX inhibitors on VEGF-induced retinal vascular leakage and experimental corneal and choroidal neovascularization. *Exp Eye Res* 2004;79:275-285.
88. Takahashi K, Saishin Y, Saishin Y, et al. Topical nepafenac inhibits ocular neovascularization. *Invest Ophthalmol Vis Sci* 2003;44:409-415.
89. Uchida N, Nagai K, Sakurada Y, et al. Distribution of VEGF and flt-1 in the normal dog tissues. *J Vet Med Sci* 2008;70:1273-1276.
90. Ambati BK, Patterson E, Jani P, et al. Soluble vascular endothelial growth factor receptor-1 contributes to the corneal antiangiogenic barrier. *Br J Ophthalmol* 2007;91:505-508.
91. Kim I, Ryan AM, Rohan R, et al. Constitutive expression of VEGF, VEGFR-1, and VEGFR-2 in normal eyes. *Invest Ophthalmol Vis Sci* 1999;40:2115-2121.

92. Kvanta A. Expression and regulation of vascular endothelial growth factor in choroidal fibroblasts. *Curr Eye Res* 1995;14:1015-1020.
93. Kociok N, Heppekausen H, Schraermeyer U, et al. The mRNA expression of cytokines and their receptors in cultured iris pigment epithelial cells: a comparison with retinal pigment epithelial cells. *Exp Eye Res* 1998;67:237-250.
94. Saint-Geniez M, Maharaj AS, Walshe TE, et al. Endogenous VEGF is required for visual function: evidence for a survival role on Muller cells and photoreceptors. *PLoS ONE* 2008;3:e3554.
95. Philipp W, Speicher L, Humpel C. Expression of vascular endothelial growth factor and its receptors in inflamed and vascularized human corneas. *Invest Ophthalmol Vis Sci* 2000;41:2514-2522.
96. Gan L, Fagerholm P, Palmblad J. Vascular endothelial growth factor (VEGF) and its receptor VEGFR-2 in the regulation of corneal neovascularization and wound healing. *Acta Ophthalmol Scand* 2004;82:557-563.
97. Tripathi RC, Li J, Tripathi BJ, et al. Increased level of vascular endothelial growth factor in aqueous humor of patients with neovascular glaucoma. *Ophthalmology* 1998;105:232-237.
98. Hu DN, Ritch R, Liebmann J, et al. Vascular endothelial growth factor is increased in aqueous humor of glaucomatous eyes. *J Glaucoma* 2002;11:406-410.
99. Vinores SA, Kuchle M, Mahlow J, et al. Blood-ocular barrier breakdown in eyes with ocular melanoma. A potential role for vascular endothelial growth factor/vascular permeability factor. *Am J Pathol* 1995;147:1289-1297.
100. Notting IC, Missotten GS, Sijmons B, et al. Angiogenic profile of uveal melanoma. *Curr Eye Res* 2006;31:775-785.
101. Stitt AW, Simpson DA, Boocock C, et al. Expression of vascular endothelial growth factor (VEGF) and its receptors is regulated in eyes with intra-ocular tumours. *J Pathol* 1998;186:306-312.
102. Shinoda K, Ishida S, Kawashima S, et al. Comparison of the levels of hepatocyte growth factor and vascular endothelial growth factor in aqueous fluid and serum with grades of retinopathy in patients with diabetes mellitus. *Br J Ophthalmol* 1999;83:834-837.
103. Pe'er J, Shweiki D, Itin A, et al. Hypoxia-induced expression of vascular endothelial growth factor by retinal cells is a common factor in neovascularizing ocular diseases. *Lab Invest* 1995;72:638-645.
104. Murata T, Nakagawa K, Khalil A, et al. The relation between expression of vascular endothelial growth factor and breakdown of the blood-retinal barrier in diabetic rat retinas. *Lab Invest* 1996;74:819-825.
105. Matsuoka M, Ogata N, Minamino K, et al. Expression of pigment epithelium-derived factor and vascular endothelial growth factor in fibrovascular membranes from patients with proliferative diabetic retinopathy. *Jpn J Ophthalmol* 2006;50:116-120.
106. Lutty GA, McLeod DS, Merges C, et al. Localization of vascular endothelial growth factor in human retina and choroid. *Arch Ophthalmol* 1996;114:971-977.
107. Vinores SA, Youssri AI, Luna JD, et al. Upregulation of vascular endothelial growth factor in ischemic and non-ischemic human and experimental retinal disease. *Histol Histopathol* 1997;12:99-109.

108. Potti A, Ganti AK, Foster H, et al. Immunohistochemical detection of HER-2/neu, c-kit (CD117) and vascular endothelial growth factor (VEGF) overexpression in soft tissue sarcomas. *Anticancer Res* 2004;24:333-337.
109. Potti A, Moazzam N, Tendulkar K, et al. Immunohistochemical determination of vascular endothelial growth factor (VEGF) overexpression in malignant melanoma. *Anticancer Res* 2003;23:4023-4026.
110. Eisma RJ, Spiro JD, Kreutzer DL. Vascular endothelial growth factor expression in head and neck squamous cell carcinoma. *Am J Surg* 1997;174:513-517.
111. Hui EP, Chan AT, Pezzella F, et al. Coexpression of hypoxia-inducible factors 1alpha and 2alpha, carbonic anhydrase IX, and vascular endothelial growth factor in nasopharyngeal carcinoma and relationship to survival. *Clin Cancer Res* 2002;8:2595-2604.
112. Toi M, Bando H, Ogawa T, et al. Significance of vascular endothelial growth factor (VEGF)/soluble VEGF receptor-1 relationship in breast cancer. *Int J Cancer* 2002;98:14-18.
113. Jebreel A, England J, Bedford K, et al. Vascular endothelial growth factor (VEGF), VEGF receptors expression and microvascular density in benign and malignant thyroid diseases. *Int J Exp Pathol* 2007;88:271-277.
114. Poon RT, Fan ST, Wong J. Clinical implications of circulating angiogenic factors in cancer patients. *J Clin Oncol* 2001;19:1207-1225.
115. Poon RT, Ho JW, Tong CS, et al. Prognostic significance of serum vascular endothelial growth factor and endostatin in patients with hepatocellular carcinoma. *Br J Surg* 2004;91:1354-1360.
116. Hyodo I, Doi T, Endo H, et al. Clinical significance of plasma vascular endothelial growth factor in gastrointestinal cancer. *Eur J Cancer* 1998;34:2041-2045.
117. Etto L, Lacerda E, Baiocchi O, et al. Clinical correlations and prognostic relevance of HGF, VEGF AND FGF expression in Brazilian patients with non-Hodgkin lymphoma. *Leuk Lymphoma* 2008;49:257-264.
118. Rossmeisl JH, Duncan RB, Huckle WR, et al. Expression of vascular endothelial growth factor in tumors and plasma from dogs with primary intracranial neoplasms. *Am J Vet Res* 2007;68:1239-1245.
119. Platt SR, Scase TJ, Adams V, et al. Vascular endothelial growth factor expression in canine intracranial meningiomas and association with patient survival. *J Vet Intern Med* 2006;20:663-668.
120. Dickinson PJ, Sturges BK, Higgins RJ, et al. Vascular endothelial growth factor mRNA expression and peritumoral edema in canine primary central nervous system tumors. *Vet Pathol* 2008;45:131-139.
121. Qiu CW, Lin DG, Wang JQ, et al. Expression and significance of PTEN and VEGF in canine mammary gland tumours. *Vet Res Commun* 2008;32:463-472.
122. Millanta F, Silvestri G, Vaselli C, et al. The role of vascular endothelial growth factor and its receptor Flk-1/KDR in promoting tumour angiogenesis in feline and canine mammary carcinomas: a preliminary study of autocrine and paracrine loops. *Res Vet Sci* 2006;81:350-357.
123. Maiolino P, De Vico G, Restucci B. Expression of vascular endothelial growth factor in basal cell tumours and in squamous cell carcinomas of canine skin. *J Comp Pathol* 2000;123:141-145.

124. Al-Dissi AN, Haines DM, Singh B, et al. 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.
125. Patruno R, Arpaia N, Gadaleta CD, et al. VEGF concentration from plasma activated platelets rich correlates with microvascular density and grading in canine mast cell tumour spontaneous model. *J Cell Mol Med* 2008.
126. Wolfesberger B, Guija de Arespacohaga A, Willmann M, et al. Expression of vascular endothelial growth factor and its receptors in canine lymphoma. *J Comp Pathol* 2007;137:30-40.
127. Yonemaru K, Sakai H, Murakami M, et al. Expression of vascular endothelial growth factor, basic fibroblast growth factor, and their receptors (flt-1, flk-1, and flg-1) in canine vascular tumors. *Vet Pathol* 2006;43:971-980.
128. Rebuzzi L, Willmann M, Sonneck K, et al. Detection of vascular endothelial growth factor (VEGF) and VEGF receptors Flt-1 and KDR in canine mastocytoma cells. *Vet Immunol Immunopathol* 2007;115:320-333.
129. Gentilini F, Calzolari C, Turba ME, et al. 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* 2005;29:1263-1269.
130. Troy GC, Huckle WR, Rossmeisl JH, et al. Endostatin and vascular endothelial growth factor concentrations in healthy dogs, dogs with selected neoplasia, and dogs with nonneoplastic diseases. *J Vet Intern Med* 2006;20:144-150.
131. Clifford CA, Hughes D, Beal MW, et al. Vascular endothelial growth factor concentrations in body cavity effusions in dogs. *J Vet Intern Med* 2002;16:164-168.
132. Clifford CA, Hughes D, Beal MW, et al. Plasma vascular endothelial growth factor concentrations in healthy dogs and dogs with hemangiosarcoma. *J Vet Intern Med* 2001;15:131-135.
133. Kato Y, Asano K, Mogi T, et al. Clinical significance of circulating vascular endothelial growth factor in dogs with mammary gland tumors. *J Vet Med Sci* 2007;69:77-80.
134. Wergin MC, Roos M, Inteeworn N, et al. The influence of fractionated radiation therapy on plasma vascular endothelial growth factor (VEGF) concentration in dogs with spontaneous tumors and its impact on outcome. *Radiother Oncol* 2006;79:239-244.
135. Carvalho JF, Blank M, Shoenfeld Y. Vascular endothelial growth factor (VEGF) in autoimmune diseases. *J Clin Immunol* 2007;27:246-256.
136. Sekiya M, Ohwada A, Miura K, et al. Serum vascular endothelial growth factor as a possible prognostic indicator in sarcoidosis. *Lung* 2003;181:259-265.
137. Malemud CJ. Growth hormone, VEGF and FGF: involvement in rheumatoid arthritis. *Clin Chim Acta* 2007;375:10-19.
138. Ciprandi G, Murdaca G, Colombo BM, et al. Serum vascular endothelial growth factor in allergic rhinitis and systemic lupus erythematosus. *Hum Immunol* 2008;69:510-512.
139. Hamada H, Ebata R, Higashi K, et al. Serum vascular endothelial growth factor in cyanotic congenital heart disease functionally contributes to endothelial cell kinetics in vitro. *Int J Cardiol* 2007;120:66-71.

140. Hamamichi Y, Ichida F, Yu X, et al. Neutrophils and mononuclear cells express vascular endothelial growth factor in acute Kawasaki disease: its possible role in progression of coronary artery lesions. *Pediatr Res* 2001;49:74-80.
141. Ray L, Mathieu M, Jespers P, et al. Early increase in pulmonary vascular reactivity with overexpression of endothelin-1 and vascular endothelial growth factor in canine experimental heart failure. *Exp Physiol* 2008;93:434-442.
142. Dunst J, Becker A, Lautenschlager C, et al. Anemia and elevated systemic levels of vascular endothelial growth factor (VEGF). *Strahlenther Onkol* 2002;178:436-441.
143. Bidder M, Towler DA, Gelberman RH, et al. Expression of mRNA for vascular endothelial growth factor at the repair site of healing canine flexor tendon. *J Orthop Res* 2000;18:247-252.
144. Park BW, Kim JR, Lee JH, et al. Expression of nerve growth factor and vascular endothelial growth factor in the inferior alveolar nerve after distraction osteogenesis. *Int J Oral Maxillofac Surg* 2006;35:624-630.
145. Vinores SA, Chan CC, Vinores MA, et al. Increased vascular endothelial growth factor (VEGF) and transforming growth factor beta (TGFbeta) in experimental autoimmune uveoretinitis: upregulation of VEGF without neovascularization. *J Neuroimmunol* 1998;89:43-50.
146. Paroli MP, Teodori C, D'Alessandro M, et al. Increased vascular endothelial growth factor levels in aqueous humor and serum of patients with quiescent uveitis. *Eur J Ophthalmol* 2007;17:938-942.
147. Aiello LP, Avery RL, Arrigg PG, et al. Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. *N Engl J Med* 1994;331:1480-1487.
148. Salom D, Diaz-Llopis M, Garcia-Delpech S, et al. Aqueous humor levels of vascular endothelial growth factor in retinitis pigmentosa. *Invest Ophthalmol Vis Sci* 2008;49:3499-3502.
149. Fine HF, Baffi J, Reed GF, et al. Aqueous humor and plasma vascular endothelial growth factor in uveitis-associated cystoid macular edema. *Am J Ophthalmol* 2001;132:794-796.
150. Missotten GS, Notting IC, Schlingemann RO, et al. Vascular endothelial growth factor a in eyes with uveal melanoma. *Arch Ophthalmol* 2006;124:1428-1434.
151. Tu KL, Kaye SB, Sidaras G, et al. Effect of intraocular surgery and ketamine on aqueous and serum cytokines. *Mol Vis* 2007;13:1130-1137.
152. Ogata N, Nishikawa M, Nishimura T, et al. Inverse levels of pigment epithelium-derived factor and vascular endothelial growth factor in the vitreous of eyes with rhegmatogenous retinal detachment and proliferative vitreoretinopathy. *Am J Ophthalmol* 2002;133:851-852.
153. Shinoda K, Ishida S, Kawashima S, et al. Clinical factors related to the aqueous levels of vascular endothelial growth factor and hepatocyte growth factor in proliferative diabetic retinopathy. *Curr Eye Res* 2000;21:655-661.
154. Patel JI, Tombran-Tink J, Hykin PG, et al. Vitreous and aqueous concentrations of proangiogenic, antiangiogenic factors and other cytokines in diabetic retinopathy patients with macular edema: Implications for structural differences in macular profiles. *Exp Eye Res* 2006;82:798-806.

155. Sahin A, Kiratli H, Tezel GG, et al. Expression of vascular endothelial growth factor a, matrix metalloproteinase 9 and extravascular matrix patterns in iris and ciliary body melanomas. *Ophthalmic Res* 2007;39:40-44.
156. Boyd SR, Tan D, Bunce C, et al. Vascular endothelial growth factor is elevated in ocular fluids of eyes harbouring uveal melanoma: identification of a potential therapeutic window. *Br J Ophthalmol* 2002;86:448-452.
157. Tolentino MJ, Miller JW, Gragoudas ES, et al. Vascular endothelial growth factor is sufficient to produce iris neovascularization and neovascular glaucoma in a nonhuman primate. *Arch Ophthalmol* 1996;114:964-970.
158. Ohno-Matsui K, Hirose A, Yamamoto S, et al. Inducible expression of vascular endothelial growth factor in adult mice causes severe proliferative retinopathy and retinal detachment. *Am J Pathol* 2002;160:711-719.
159. Ishida S, Usui T, Yamashiro K, et al. VEGF164-mediated inflammation is required for pathological, but not physiological, ischemia-induced retinal neovascularization. *J Exp Med* 2003;198:483-489.
160. Su CY, Chen MT, Wu WS, et al. Concentration of vascular endothelial growth factor in the subretinal fluid of retinal detachment. *J Ocul Pharmacol Ther* 2000;16:463-469.
161. Adamis AP, Shima DT, Tolentino MJ, et al. Inhibition of vascular endothelial growth factor prevents retinal ischemia-associated iris neovascularization in a nonhuman primate. *Arch Ophthalmol* 1996;114:66-71.
162. Deeg CA, Altmann F, Hauck SM, et al. Down-regulation of pigment epithelium-derived factor in uveitic lesion associates with focal vascular endothelial growth factor expression and breakdown of the blood-retinal barrier. *Proteomics* 2007;7:1540-1548.
163. Mason JO, 3rd, Albert MA, Jr., Mays A, et al. Regression of neovascular iris vessels by intravitreal injection of bevacizumab. *Retina* 2006;26:839-841.
164. Davidorf FH, Mouser JG, Derick RJ. Rapid improvement of rubeosis iridis from a single bevacizumab (Avastin) injection. *Retina* 2006;26:354-356.
165. Chilov MN, Grigg JR, Playfair TJ. Bevacizumab (Avastin) for the treatment of neovascular glaucoma. *Clin Experiment Ophthalmol* 2007;35:494-496.
166. Wakabayashi T, Oshima Y, Sakaguchi H, et al. Intravitreal bevacizumab to treat iris neovascularization and neovascular glaucoma secondary to ischemic retinal diseases in 41 consecutive cases. *Ophthalmology* 2008;115:1571-1580, 1580 e1571-1573.
167. Foy JW, Rittenhouse K, Modi M, et al. Local tolerance and systemic safety of pegaptanib sodium in the dog and rabbit. *J Ocul Pharmacol Ther* 2007;23:452-466.
168. Manzano RP, Peyman GA, Khan P, et al. Testing intravitreal toxicity of bevacizumab (Avastin). *Retina* 2006;26:257-261.
169. Bakri SJ, Cameron JD, McCannel CA, et al. Absence of histologic retinal toxicity of intravitreal bevacizumab in a rabbit model. *Am J Ophthalmol* 2006;142:162-164.
170. Luthra S, Narayanan R, Marques LE, et al. Evaluation of in vitro effects of bevacizumab (Avastin) on retinal pigment epithelial, neurosensory retinal, and microvascular endothelial cells. *Retina* 2006;26:512-518.
171. Bakri SJ, Snyder MR, Reid JM, et al. Pharmacokinetics of intravitreal ranibizumab (Lucentis). *Ophthalmology* 2007;114:2179-2182.

172. Peters S, Heiduschka P, Julien S, et al. Immunohistochemical localisation of intravitreally injected bevacizumab in the anterior chamber angle, iris and ciliary body of the primate eye. *Br J Ophthalmol* 2008;92:541-544.
173. Bakri SJ, Larson TA, Edwards AO. Intraocular inflammation following intravitreal injection of bevacizumab. *Graefes Arch Clin Exp Ophthalmol* 2008;246:779-781.
174. Bakri SJ, Patel SP. Retinal pigment epithelial tear following intravitreal bevacizumab. *Eye* 2007;21:424-425.
175. Kamstock D, Elmslie R, Thamm D, et al. Evaluation of a xenogeneic VEGF vaccine in dogs with soft tissue sarcoma. *Cancer Immunol Immunother* 2007;56:1299-1309.
176. Williams GA, Eisenstein R, Schumacher B, et al. Inhibitor of vascular endothelial cell growth in the lens. *Am J Ophthalmol* 1984;97:366-371.
177. Somnath B, Vijay S, Scott RAH, et al. Multiplex bead analysis of vitreous humor of patients with vitreoretinal disorders. *Investigative Ophthalmology & Visual Science* 2007;48:2203-2207.
178. Dong A, Xie B, Shen J, et al. Oxidative stress promotes ocular neovascularization. *J Cell Physiol* 2009;219:544-552.

APPENDIX

Table 1: Case distribution

Disease process	Stage or type	Aqueous samples	Plasma samples	# Dogs
Normal		26	0	13
Primary cataract		44	20	32
	Incomplete	*14*	*3*	*10*
	Complete	*13*	*7*	*9*
	Resorbing	*17*	*10*	*13*
Diabetic cataract		51	17	30
	Incomplete	*9*	*4*	*5*
	Complete	*32*	*11*	*19*
	Resorbing	*10*	*2*	*6*
Aphakic/pseudophakic glaucoma		7	6	7
Lens-induced uveitis		18	6	13
Primary glaucoma		41	14	35
Uveitic glaucoma		24	15	23
Retinal detachment		13	6	11
Tumor		19	12	19
	Benign melanoma	*8*	*3*	*8*
	Malignant melanoma	*4*	*3*	*4*
	Adenoma	*4*	*3*	*4*
Lens instability		7	3	6
Ocular melanosis		1	1	1
Uveal cyst syndrome		1	1	1
Total		252	101	191

Table 2: Mean Aqueous Humor Concentrations of VEGF

Disease process	Stage or type	Number	Mean $[VEGF]_{AH}$ in pg/mL	95% confidence interval
Normal		26	10.6	2.9-39.0
Primary cataract		44	28.2	9.2-86.3
	Incomplete	*14*	*17.9*	*1.0-262*
	Complete	*13*	*46.1*	*3.3-642*
	Resorbing	*17*	*14.9*	*0-248*
Diabetic cataract		51	45	14.7-138
	Incomplete	*9*	*24.3*	*3.2-190*
	Complete	*32*	*45.6*	*5.3-394*
	Resorbing	*10*	*37.2*	*5.0-277*
Aphakic/pseudophakic glaucoma		7	496	100.0-2460
Lens-induced uveitis		18	665	187-2360
Primary glaucoma		41	601	198-1830
Uveitic glaucoma		24	2150	686-6710
Retinal detachment		13	3120	816-11900
Tumor		19	1400	429-4540
	Benign melanoma	*8*	*251*	*12.7-4950*
	Malignant melanoma	*4*	*1040*	*31.1-35000*
	Adenoma	*4*	*11850*	*362-38800*
Lens instability		7	1030	187-5653
Ocular melanosis		1	1253	Not applicable
Uveal cyst syndrome		1	814.6	Not applicable

Table 3: Distribution of PIFMs in Histopathology Samples

Disease	Type	Number	Absent (0)	Cellular (1)	Vascular or fibrous (2)	Fibrovascular (3)
Aphakic/pseudophakic glaucoma		4	1	2	1	0
Primary glaucoma		18	8	7	2	1
Uveitic glaucoma		19	5	7	2	5
Retinal detachment		8	1	2	2	3
Tumor		18	2	3	7	6
	Benign melanoma	*8*	*1*	*1*	*4*	*2*
	Malignant melanoma	*3*	*1*	*0*	*1*	*1*
	Adenoma	*4*	*0*	*1*	*2*	*1*
Lens instability		4	0	3	1	0
Ocular melanosis		1	0	1	0	0
Uveal cyst syndrome		1	0	1	0	0
Total		73	17	26	15	15

Table 4: Aqueous Humor and Plasma VEGF Concentration for Samples Within the Limits of Detection and Below the Limits of Detection.

		Plasma VEGF		
		Below limits of detection	Within limits of detection	
Aqueous Humor VEGF	Below limits of detection	(n = 8) OD: 3 OS: 5 Mean$[VEGF]_{AH}$ =TLTM Mean$[VEGF]_{PL}$ = TLTM	(n = 2) OD: 1 OS: 1 Mean$[VEGF]_{AH}$ = TLTM Mean$[VEGF]_{PL}$ = 40pg/mL	10
	Within limits of detection	(n = 46) OD: 27 OS: 19 Mean$[VEGF]_{AH}$ = 500pg/mL Mean$[VEGF]_{PL}$ = TLTM	(n = 66) OD: 32 OS: 34 Mean$[VEGF]_{AH}$ = 445pg/mL Mean$[VEGF]_{PL}$ = 19.1pg/mL	112
		54	68	Total: 122

Key:
OD = right eye, OS = left eye
$[VEGF]_{AH}$ = Vascular endothelial growth factor concentration in aqueous humor
$[VEGF]_{PL}$ = Vascular endothelial growth factor concentration in plasma
TLTM = too low to measure

Figure 1: Scatter Plot of Plasma VEGF and Aqueous Humor VEGF Concentration

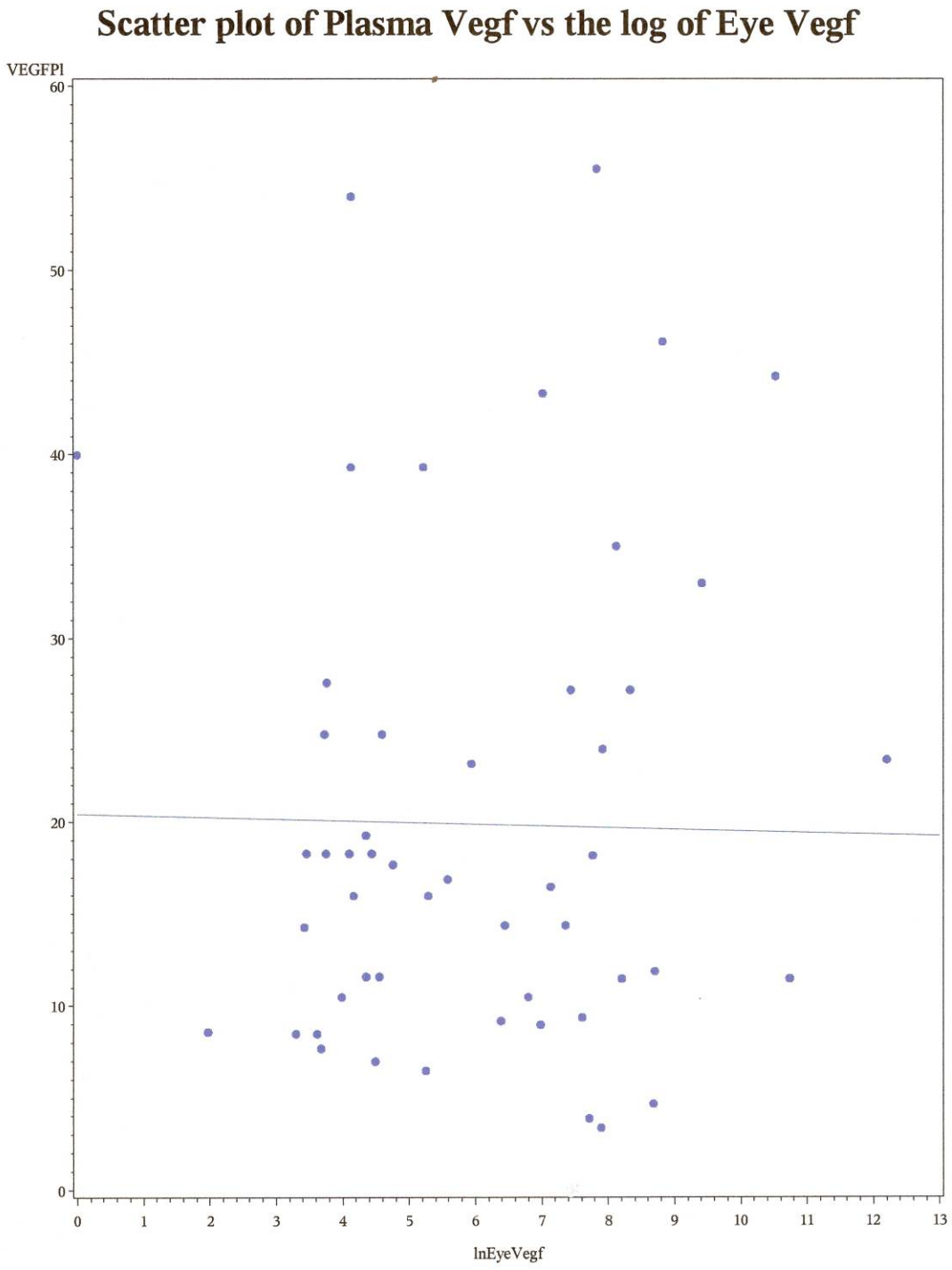


Figure 2: Bar Graph of Mean Aqueous Humor VEGF Concentration by Disease. Significant difference of disease groups from dogs without intraocular disease is denoted with an asterisk (*).

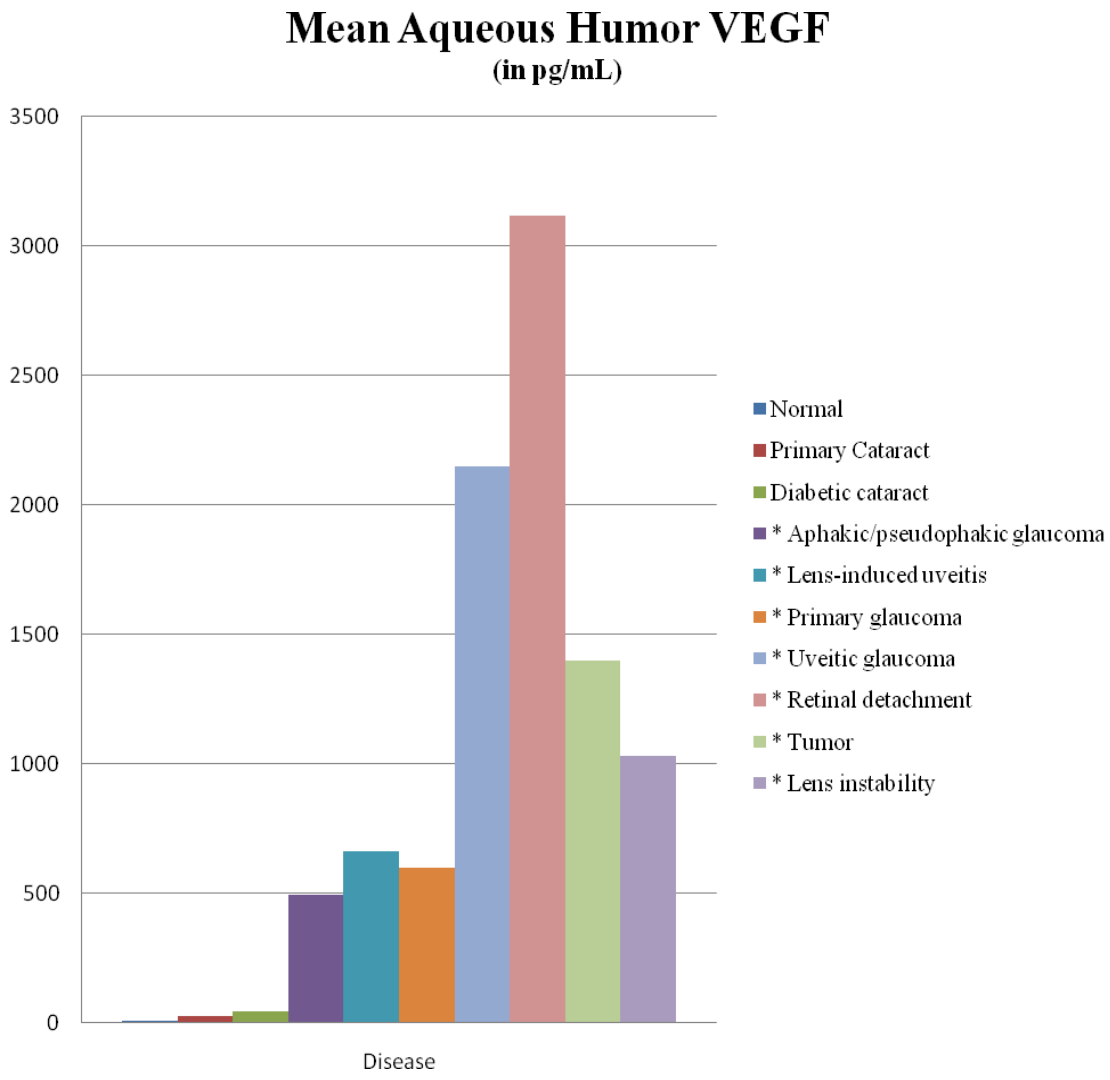


Figure 3: Cellular PIFM at 40x magnification. A cellular layer lacking blood vessels covers the anterior iris epithelium. The arrow indicates the interface of the anterior iris and cellular membrane.

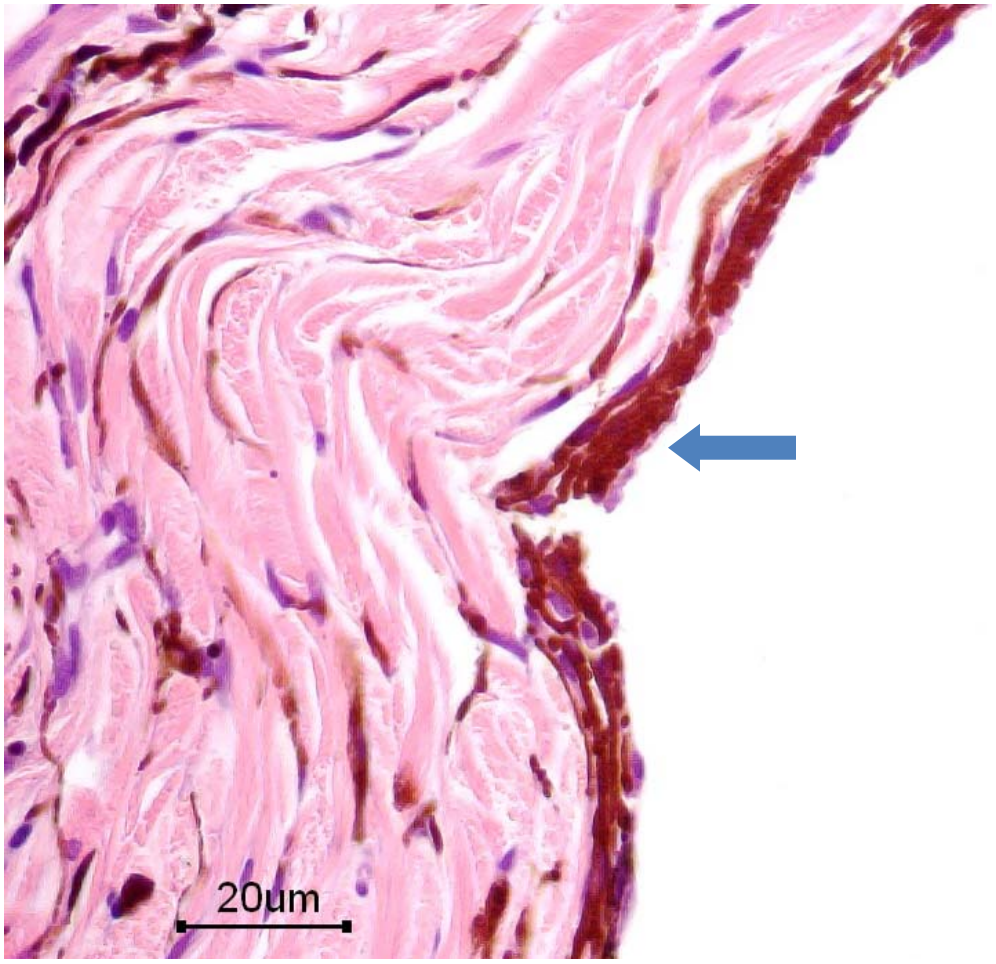


Figure 4: Vascular PIFM at 40x magnification. A thin cellular layer with blood vessels covers the anterior iris epithelium. A blood vessel is indicated by the arrow in this view.

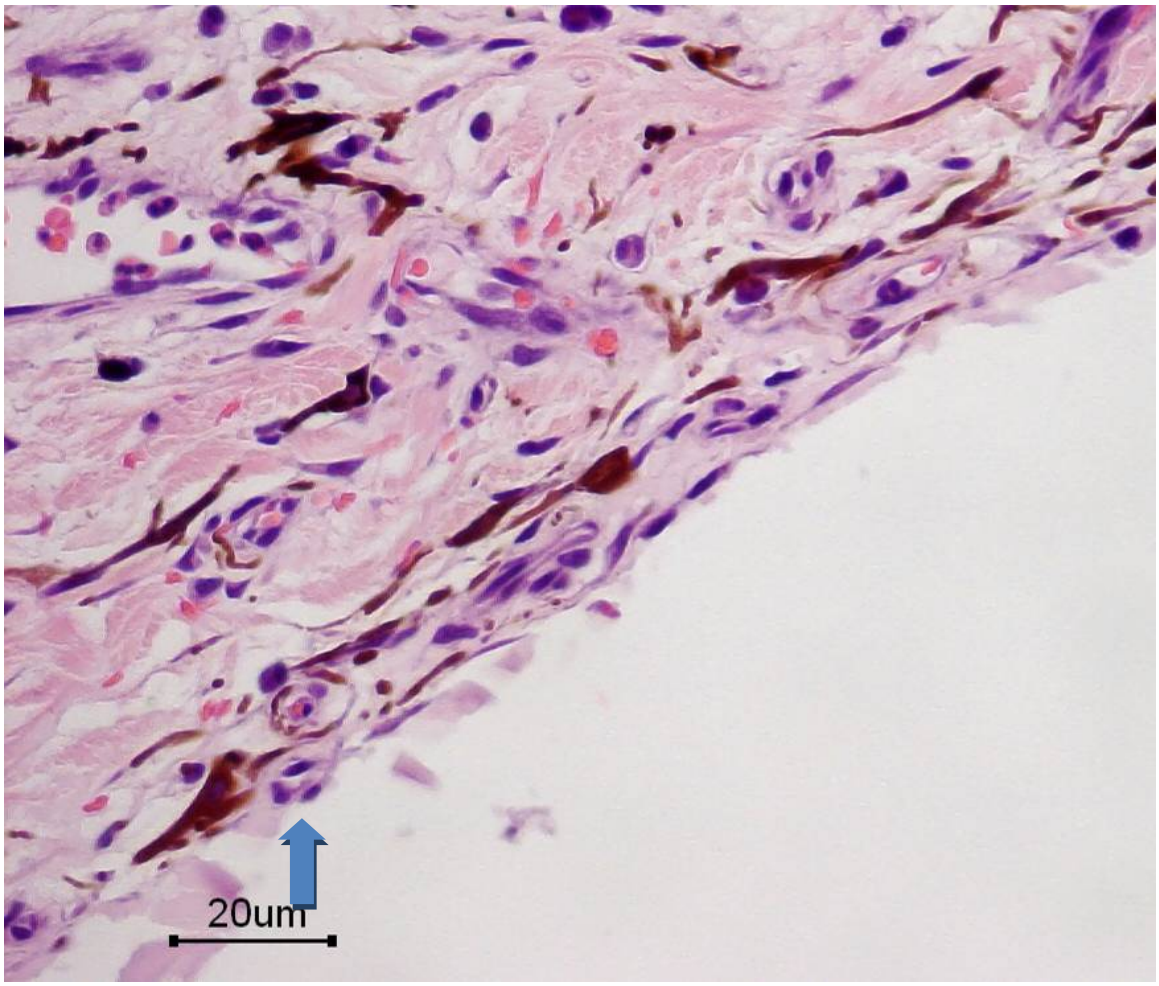


Figure 5: Fibrovascular PIFM at 4x magnification. A thick fibrovascular membrane is covering the anterior surface of the iris and causing entropion uvea, as indicated by the arrow.

Figure 6: Fibrovascular membrane at 10x magnification. The membrane is extending over the iridocorneal angle (indicated by arrow).

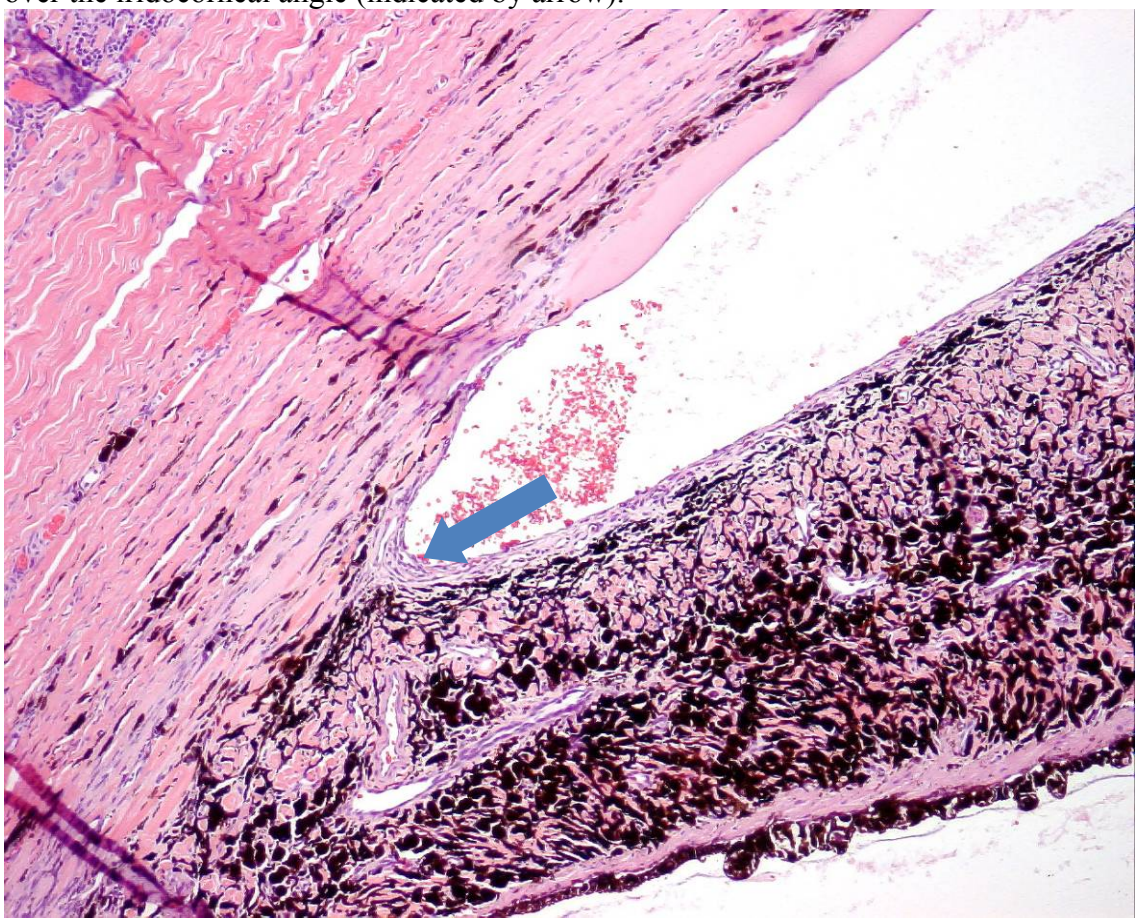
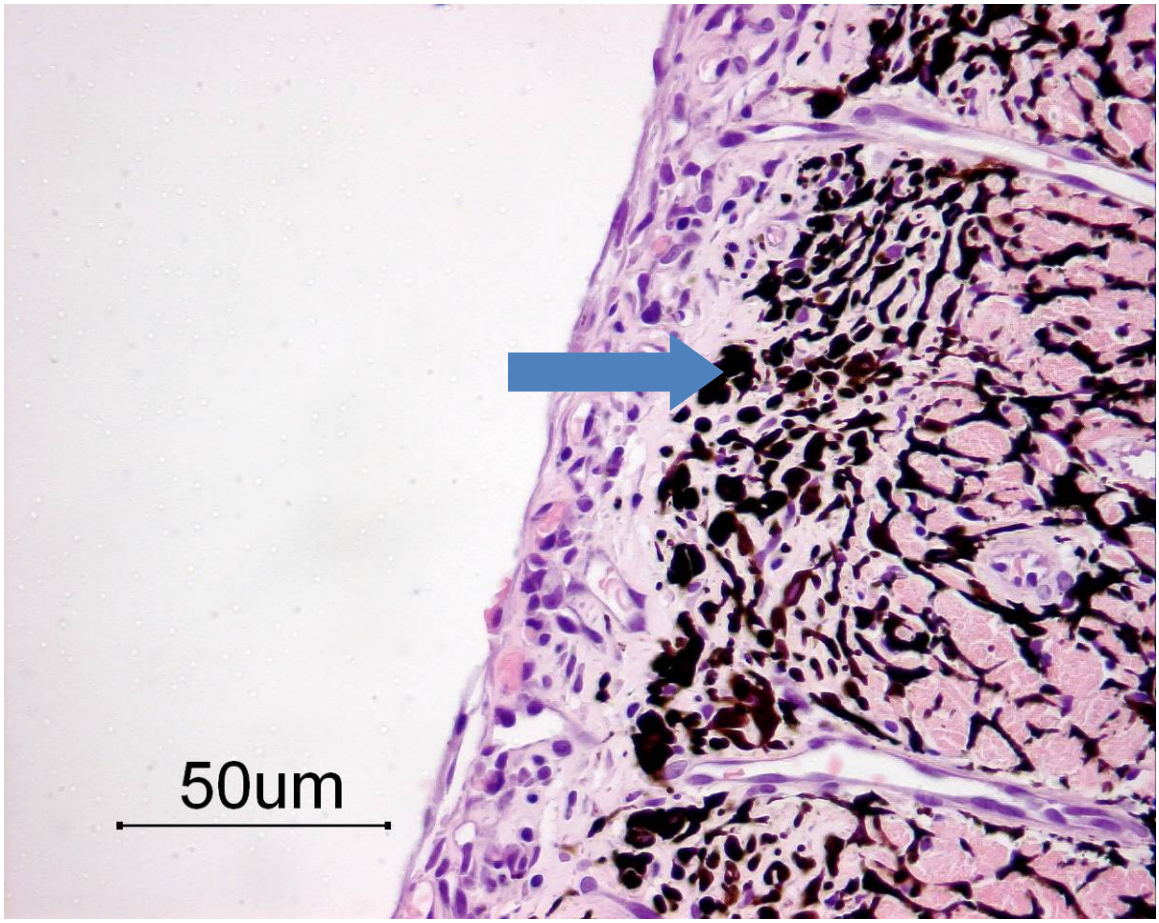


Figure 7: Fibrovascular PIFM at 40x magnification. Evaluation of the structure at this magnification reveals blood vessels and connective tissue covering the anterior iris. The arrow indicates the junction of the anterior iris with the fibrovascular membrane.



Sample summary: "patient" refers to patient identification number; "OD", "OS" and "Pl" refer to the VEGF concentration of the right eye, left eye and plasma, respectively; "ocular disease" refers to ocular disease (1, 2 and 3 refer to incomplete, complete and resorbing cataract, respectively); "age" refer to age in years; "sex" refers to gender (M = intact male, MC= castrated male, F= intact female, FS= spayed female); "duration" refers to ocular disease duration in months; "PIFM" refers to pre-iridal fibrovascular membrane (0=not present; 1= cellular, 2= vascular or fibrous, 3= fibrovascular).

Patient	OD	OS	Pl	Ocular Disease	Age	Breed	Sex	Systemic Disease	Duration	Medications	PIFM	Glaucoma
DMCR	0	24.3		Control	2	Mixed	M	None		None		No
IMCR	10.3	19.5		Control	2	Mixed	M	None		None		No
LMEK	63	39.7		Control	2	Mixed	M	None		None		No
MAJ	12.4	14.5		Control	2	Mixed	M	None		None		No
MAP	13.7	7.9		Control	2	Mixed	M	None		None		No
MAR	12.5	0		Control	2	Mixed	M	None		None		No
MAY	10.1	12		Control	2	Mixed	M	None		None		No
MB5	9.5	0		Control	2	Mixed	M	None		None		No
MBI	64.5	49.7		Control	2	Mixed	M	None		None		No
MBJ	7.4	11.2		Control	2	Mixed	M	None		None		No
MBY	0	0		Control	2	Mixed	M	None		None		No
MCX	14.4	16.5		Control	2	Mixed	M	None		None		No
MFY	25.2	5		Control	2	Mixed	M	None		None		No
95874		28.6		Primary cataract- 1	6	Basset Hound	MC	None	7	Topical, systemic steroid		No
99213	48.6	191		Primary cataract- 1	7	Beagle	MC	None	12	Topical, systemic steroid		No
92685	147	37.3	0	Primary cataract- 2	13	Bichon Frise	FS	Atopy	0.25	Topical steroid	0	No
93064	0		0	Primary cataract- 1	10	Bichon Frise	FS	None	18	Topical steroid		No
AM3A	36.4	26.1	8.5	Primary cataract- 2	8	Bichon Frise	MC	None	3	Topical steroid, prostaglandin		No
97313	131	163		Primary cataract- 1	11	Boston Terrier	FS	None	10	Topical		No

97816		155		Primary cataract-	1	11	Boston Terrier	FS	Atopy	8	Topical	No
99268	63.5	198	16	Primary cataract-	3	7	Boston Terrier	FS	None	18	Topical	No
99918	0	15.5		Primary cataract-	3	2	Cocker Spaniel	F	None	2	Topical	No
AM2A	6.2		8.6	Primary cataract-	2	2	Cocker Spaniel	FS	None	3	Topical steroid, prostaglandin	No
SA3A	0		0	Primary cataract-	1	2	Cocker Spaniel	MC	None	2	Topical steroid	No
91135		0		Primary cataract-	3	7	JRT	MC	None	30	Topical, systemic steroid	No
97546		17.1	0	Primary cataract-	3	1	JRT	FS	Atopy	3.5	Topical, systemic steroid	No
97146	42.3	29.7		Primary cataract-	3	6	Other	FS	None	0.75	Topical, systemic steroid	No
98474	189.3	12.3		Primary cataract-	1	13	Other	FS	HAC	12	Topical, systemic steroid	No
91439		54.4		Primary cataract-	2	6	Other	FS	Other	1.5	Topical steroid	No
68171		0	0	Primary cataract-	3	11	Mixed	FS	None	2	Topical, systemic steroid	No
AM1A		191	6.5	Primary cataract-	2	14	Mixed	MC	None	3	Topical steroid	No
SA11A	31.6			Primary cataract-	1	4	Mixed	MC	None		Topical steroid	No
SA5A	553		0	Primary cataract-	2	3.5	Mixed	MC	None	2	Topical steroid	No
100058	179	5.3		Primary cataract-	2	6	Other	FS	None	6	Topical, systemic steroid	No
88313	42.2		27.6	Primary cataract-	3	8	Other	MC	None	24	Topical, systemic steroid	No
97029		38.6	7.7	Primary cataract-	1	6	Other	FS	None	6	Topical, systemic steroid	No
99975	5.1	51.4		Primary cataract-	1	11	Other	MC	None	12	Topical, systemic steroid	No

96844		116	17.7	Primary cataract-	2	6	Other	MC	None	2	Topical, systemic steroid	No
99247		89	0	Primary cataract-	3	10	Other	MC	None	18	Topical, systemic steroid	No
92855	31.6	0	0	Primary cataract-	2	11	Pug	MC	None	8.5	Topical steroid	No
BM14A		24.3	0	Primary cataract-	3	8	Other	MC	None		Topical steroid	No
BM16A	26.9		0	Primary cataract-	3	13	Other	MC	None	24	None	No
93519	0	0	0	Primary cataract-	3	8	Other	FS	None	12	Topical, systemic steroid	No
BM12A	721		0	Primary cataract-	3	7.5	Other	MC	None	6	None	No
BM7A	381		23.2	Primary cataract-	2	7	Other	FS	None	0.5	None	No
92451		94.7	0	Diabetic cataract-	3	6	Beagle	FS	DM	3	Topical steroid	No
95939	77	94.2	11.6	Diabetic cataract-	2	10	Bichon Frise	FS	DM	3.5	Topical steroid	No
98081	24.3	31.8		Diabetic cataract-	2	8	Bichon Frise	MC	DM	1	Topical steroid	No
100022	48.5	23.5		Diabetic cataract-	1	11	JRT	MC	DM	3.5	Topical steroid	No
99450	11.7	4.6	0	Diabetic cataract-	2	6	JRT	MC	DM	1.5	Topical steroid	No
89550	127			Diabetic cataract-	3	10	Lab Ret	M	DM, other	4	Topical steroid	No
94352	0	0	40	Diabetic cataract-	1	7	Lab Ret	FS	DM	0.8	Topical steroid	No
96458	88.2	89.5	0	Diabetic cataract-	3	10	Lab Ret	FS	DM	18	Topical steroid	No
96637	94.1	105		Diabetic cataract-	2	11	Lab Ret	FS	DM	12	Topical steroid	No
99714	83.7	59.7	18.3	Diabetic cataract-	2	10	Lab Ret	MC	DM	6	Topical steroid	

SA19A		45.1	0	Diabetic cataract-	2	12	Lab Ret	FS	DM		None	No
SA2A	94.2		0	Diabetic cataract-	2	13	Lab Ret	FS	DM		None	No
90840	0	0		Diabetic cataract-	2	3	Mixed	FS	DM	7	Topical steroid	No
90869	0	0		Diabetic cataract-	3	2	Mixed	M	DM	6	Topical steroid	No
90903	48	0		Diabetic cataract-	3	3	Mixed	FS	DM	4	Topical steroid	No
97702		213		Diabetic cataract-	2	8	Mixed	FS	DM	4	Topical steroid	No
98082	61.5		54	Diabetic cataract-	1	13	Mixed	FS	DM	1.5	Topical steroid	No
98303	40.8	98.5	24.8	Diabetic cataract-	1	8	Mixed	MC	DM	6	Topical steroid	No
BM10A	76.8		19.3	Diabetic cataract-		6	Mixed	MC	DM	6	None	No
BM3A	266		16.9	Diabetic cataract-	2	9	Mixed	MC	DM	3	None	No
89992	385	150		Diabetic cataract-	2	11	Other	FS	DM	10	Topical steroid	No
90931	85.6	0		Diabetic cataract-	2	5	Other	MC	DM	6	Topical steroid	No
92116	127	136	989	Diabetic cataract-	2	10	Other	FS	DM	8	Topical steroid	No
92571	21.7	30.3		Diabetic cataract-	2	9	Other	FS	DM	2	Topical steroid	No
96783	53.2	898	10.5	Diabetic cataract-	1	7	Other	FS	DM	3	Topical steroid	No
97601	124	139		Diabetic cataract-	2	6	Other	MC	DM	1	Topical steroid	No
97966	30.8	41.7	18.3	Diabetic cataract-	2	16	Other	MC	DM	5	Topical steroid	No
99281	106	66.7		Diabetic cataract-	3	10	Other	MC	DM	6	Topical steroid	No

							MC	DM				No
96434	184	61.2	39.3	Diabetic cataract- 2	5	Pug			1.5	Topical steroid		
BM2A	29.8		14.3	Diabetic cataract- 2	4	Pug	MC	DM	2	None		No
HD5A		737	0	Pseudo-phakic glaucoma	10	Bichon Frise	MC	None	1	None		Yes
81646		762	0	Aphakic glaucoma	10	Cocker Spaniel	MC	None	0.17	Systemic steroid	1	Yes
AM17A	1820		0	Pseudo-phakic glaucoma	12	JRT	MC	None	2.5	Topical steroid, prostaglandin	2	Yes
73695	695		0	Pseudo-phakic glaucoma	9	Other	MC	None	11	Topical steroid	1	Yes
SV1A		1980	0	Pseudo-phakic glaucoma	12	Other	FS	DM	3	Topical steroid		Yes
72690	7.8			Aphakic glaucoma	9	Other	FS	Cardiac	1	Systemic steroid, topical prostaglandin		Yes
HD1A	1600		0	Pseudo-phakic glaucoma	9	Other	MC	None	4	Topical steroid		Yes
92310	819	747		Lens-induced uveitis- 2	5	Beagle	FS	DM	0.25	Topical steroid		No
HD2A		1120	43.3	Lens-induced uveitis- 3	6	Cocker Spaniel	MC	None	1	Topical steroid		Yes
HDA7	595		0	Lens-induced uveitis- 3	10	Cocker Spaniel	MC	None	7	None		Yes
BM21A	1290		0	Lens-induced uveitis- 3	9	Lab Ret	MC	None	3	Topical steroid		No
91560	43.1			Lens-induced uveitis- 3	12	Other	FS	Cardiac	13	Topical steroid		Yes
98137	1780	1760	0	Lens-induced uveitis	6	Other	MC	None	6	Topical steroid	3	Yes

98575	1429	2185		Lens-induced uveitis- 2	7	Other	MC	None	0.75	Topical steroid		No
AM12891		1660		Lens-induced uveitis- 3	12	Other	MC	None	2	Topical steroid		Yes
24356	465	645		Lens-induced uveitis- 2	7	Other	M	DM	3	Topical steroid, prostaglandin	1	No
91107	1560	193		Lens-induced uveitis- 3	10	Other	MC	DM	4.5	Topical steroid		No
99668	2530		55.5	Lens-induced uveitis- 3	13	Other	FS	None	0.75	Topical steroid	0	Yes
86967	726		0	Lens-induced uveitis- 2	3	Other	MC	None	0.1	Topical, systemic steroid		Yes
PSA4	237			Lens-induced uveitis- 3	7	Other	FS	None	1	Topical steroid, prostaglandin		Yes
92615	1500			Primary glaucoma	6	Basset Hound	FS	None	0.5	None	1	Yes
99534	1050		0	Primary glaucoma	9	Basset Hound	FS	None	0.25	Topical prostaglandin	1	Yes
AM6A		2780	0	Primary glaucoma	8	Basset Hound	FS	None	3	Topical prostaglandin		Yes
BM17A		1590	0	Primary glaucoma	10	Basset Hound	FS	None	5	None	2	Yes
BM18A		2850	0	Primary glaucoma	7	Basset Hound	FS	None		None	2	Yes
BM6A	210		0	Primary glaucoma	14	Basset Hound	FS	None	18	Topical prostaglandin		Yes
AM16494	2960			Primary glaucoma	14	Cocker Spaniel	M	None	3	Topical steroid, prostaglandin		Yes
57907		26.1		Primary glaucoma	9	Cocker Spaniel	FS	Cardiac	4	Topical prostaglandin		Yes
87670		178		Primary glaucoma	1	Cocker Spaniel	M	None	7	Topical steroid	0	Yes
90374	11200			Primary glaucoma	12	Cocker Spaniel	MC	None	12	None		Yes
90949	2420	799	0	Primary glaucoma	11	Cocker Spaniel	FS	None	0.5	Topical prostaglandin	0	Yes

				Primary		Cocker	FS	None		Topical		Yes
91249	553			glaucoma	9	Spaniel			0.8	prostaglandin	0	
				Primary		Cocker				Topical		Yes
95216	1630			glaucoma	11	Spaniel	FS	None	0.1	prostaglandin	1	
				Primary		Cocker				Topical steroid,	Yes	
PSA1		2010		glaucoma	13	Spaniel	FS	Atopy	3	prostaglandin		
				Primary		Cocker		None		Topical	Yes	
PSA2		398		glaucoma	14	Spaniel	FS		2	prostaglandin		
				Primary				None		Topical steroid,		Yes
AM19A		0	0	glaucoma	11	JRT	FS		3	prostaglandin	0	
				Primary				None				Yes
HD3A	594		9.2	glaucoma	4	JRT	M		6	Topical steroid		
				Primary				None				Yes
HD4A		146	0	glaucoma	10	JRT	F		6	Topical steroid		
				Primary				None		Topical		Yes
92684	630	1570	14.4	glaucoma	8	Lab Ret	MC		0.1	prostaglandin		
				Primary				None		Topical steroid,		Yes
AM18A		6830	46.1	glaucoma	10	Lab Ret	FS		2.5	prostaglandin	0	
				Primary								Yes
69243		307		glaucoma	11	Mixed	MC	Other	1	None	3	
				Primary			FS	None		Topical		Yes
93705	191			glaucoma	10	Mixed			12	prostaglandin	0	
				Primary			FS	None				Yes
96753	5600	146		glaucoma	2	Mixed			12	None	0	
				Primary			FS	None		Topical		Yes
97866	190	53.5	0	glaucoma	10	Mixed			7	prostaglandin	1	
				Primary			FS					Yes
98792	76.1	785		glaucoma	13	Mixed		Other	1	None	1	
				Primary						Topical		Yes
MDLBRT	14500			glaucoma	5	Mixed	F	Other	0.5	prostaglandin	0	
				Primary			FS	None		Topical steroid,		Yes
SA9A		6550	0	glaucoma	9	Mixed			14	prostaglandin		
				Primary			FS	None				Yes
96546	1760			glaucoma	12	Other			2	None	1	
				Primary			FS	None		Topical		Yes
98370	125			glaucoma	12	Other			2	prostaglandin		

AM13A		32.1		Primary glaucoma	8	Other	MC	None	24	Topical steroid, prostaglandin		Yes
BM8A	37.3		0	Primary glaucoma	13.5	Other	FS	None	6	Topical steroid		Yes
91435		41900		Primary glaucoma	9	Pug	FS	None	2	Topical prostaglandin		Yes
AM27044	4930			Primary glaucoma	8	Other	FS	None	1	Topical steroid, prostaglandin		Yes
99287		263		Primary glaucoma	8	Other	FS	None	5	None	0	Yes
98274	482	183		Primary glaucoma	10	Other	FS	None	12	None		Yes
97734	519			Uveitic glaucoma	7	Beagle		Other	0.5	Topical steroid, prostaglandin	3	Yes
94914		1170	0	Uveitic glaucoma	8	Cocker Spaniel	MC	None	0.002	Topical steroid	1	Yes
95150	792		0	Uveitic glaucoma	10	Cocker Spaniel	MC	None	0.75	Topical steroid	2	Yes
96101		2760	24	Uveitic glaucoma	15	JRT	FS	None	0.5	Topical steroid	1	Yes
98944		724		Uveitic glaucoma	7	JRT	FS	None	1.5	Topical steroid	0	Yes
96519		5400		Uveitic glaucoma	6	Lab Ret	MC	None	0.5	Systemic steroid	3	Yes
BM1A		3070	0	Uveitic glaucoma	3	Other	MC	None	3	Topical steroid		Yes
SA4A	33000		0	Uveitic glaucoma	14	Other	MC	None	1.5	Topical steroid		Yes
BM13A	6040		11.9	Uveitic glaucoma	12	Other	FS	None	0.5	None	3	Yes
AM27865		786		Uveitic glaucoma	8	Mixed	FS	None		Topical steroid	2	Yes
95353	224		0	Uveitic glaucoma	8	Mixed	FS	None	0.75	Topical steroid	0	Yes
98516	4170	1704	27.2	Uveitic glaucoma	11	Mixed	FS	Other	0.75	Topical steroid	0	Yes

				Uveitic glaucoma						Topical steroid		Yes
46253	58000		0		9	Other	MC	None	0.75		1	
59386		10200		Uveitic glaucoma	9	Other	MC	DM	0.25	Topical prostaglandin	3	Yes
84804	79.6			Uveitic glaucoma	6	Other	FS	None	12	None	0	Yes
97757	705		0	Uveitic glaucoma	4	Other	FS	None	4	Topical steroid, prostaglandin		Yes
BM4A		253	0	Uveitic glaucoma	4	Other	F	Other	2	Topical steroid	1	Yes
EC2A		3390	35	Uveitic glaucoma	8	Other	FS	None	9	Topical steroid, prostaglandin	0	Yes
HD6A	47400		0	Uveitic glaucoma	10	Pug	MC	None	2	Topical steroid		Yes
89181		869		Uveitic glaucoma	8	Other	FS	None	18	None	1	Yes
96065		37400	44.2	Uveitic glaucoma	10	Other	MC	DM	0.3	None	3	
98783	1942			Uveitic glaucoma	9	Other	FS	None	1	Topical steroid	1	No
98273		304	0	Uveitic glaucoma	2	Other	MC	Other	0.2	Topical steroid	2	Yes
99547		12550		Retinal detachment	4	Beagle	FS	None	2	Topical steroid	1	No
AM28242		3880		Retinal detachment	4	Boston Terrier	MC	None	0.5	Topical steroid, prostaglandin	2	No
86366		257		Retinal detachment	6	Boston Terrier	MC	None	5	Topical steroid, prostaglandin		No
90493	71.8	102	0	Retinal detachment	5	Boston Terrier	MC	None	0.5	None	1	No
SA20A	41800			Retinal detachment	6	Cocker Spaniel	FS	None	6	Systemic steroid		No
SA6A		2370	18.2	Retinal detachment	6	Cocker Spaniel	FS	None	1	Systemic steroid		No
97815	26500	6790		Retinal detachment	0.75	Lab Ret	M		9	None	2	

96086	1080		9	Retinal detachment	6	Other	MC	None	2.5	None	3	No
98258	1600		0	Retinal detachment	6	Mixed	FS	None	0.001	None	0	No
AM16A		20300	0	Retinal detachment	11	Mixed	MC	None		Topical steroid, prostaglandin	3	No
90350	12300		33	Retinal detachment	10	Other	M	None	24	Topical steroid	3	No
99051	0			Benign melanoma	2	Beagle	FS	None	0.001	None	1	No
SA1A		200000	23.4	Malignant melanoma	12	Beagle	FS	None	6	Topical prostaglandin		Yes
95822	1260		16.5	Benign melanoma	10	Other	FS	None	1	Topical steroid	3	No
EC3A	5910		4.7	Adenoma	7	Other	FS	None		None	2	Yes
94941		2020	9.4	Benign melanoma	10	JRT	FS	None	6	Topical steroid	2	No
100556		16300	0	Malignant melanoma	12	Lab Ret	FS	None	1	None	3	No
90375	270			Benign melanoma	10	Lab Ret	FS	None	2	Topical steroid	2	No
92739		46100	11.5	Adenoma	9	Lab Ret	MC	None	0.25	Topical prostaglandin	2	No
92779		343		Malignant melanoma	8	Lab Ret	F	None	0.24	None	2	No
100175		0	0	Malignant melanoma	7	Mixed	MC	None	3.5	None	0	No
93911		88.7	7.0	Benign melanoma	8	Mixed	FS	None	30	None	2	Yes
94907	2700		3.4	Spindle cell tumor	8	Mixed	MC	Other	0.5	Topical steroid, prostaglandin	3	Yes
99666		281		Benign melanoma	13	Mixed	FS	None	1	Topical steroid	0	Yes
EC1A	63400		0	Anaplastic	13	Mixed	FS	None	1.5	Topical steroid, prostaglandin	3	Yes
100276	33500			Adenoma	8	Other	FS	None	0.5	None	3	No

AM25917	1060			Benign melanoma	12	Other	MC	None	2	Topical steroid, prostaglandin	3	No
90742	788			Benign melanoma	13	Other	MC	None	2.5	Topical steroid	2	No
98346		3670	11.5	Lymphoma	8	Other	FS	None	0.5	Topical steroid	1	Yes
AM9A		2250	3.9	Adenoma	11	Other	FS	None	3	Topical steroid	1	No
79800	475	80.7	0	Lens luxation	6	Beagle	FS	Hypertension	2.5	Topical steroid, prostaglandin	1	Yes
91853		116		Lens luxation	6	JRT	FS	None	0.001	None		Yes
96105	2570		0	Lens luxation	16	Mixed	FS	None	0.5	None	1	Yes
77820	2230		0	Lens luxation	7	Other	MC	None	10	Topical prostaglandin	1	Yes
91584		2530		Lens luxation	3	Other	F	None	0.5	Topical prostaglandin	2	Yes
28594		684		Lens luxation	18	Other	MC	None	0.5	Topical steroid		Yes