UNIVERSIDADE DE LISBOA

Faculdade de Medicina Veterinária

EFFECTS OF METHADONE ON INTRAOCULAR PRESSURE IN DOGS AND CATS

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DISSERTAÇÃO DE MESTRADO INTEGRADO EM MEDICINA VETERINÁRIA

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2018

Lisboa

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In memory of Manuel José Pinto and Mike, whose spirits guide me every single day

Acknowledgments

My words of acknowledgment are mostly towards my thesis advisor Professor Esmeralda Delgado for having her office door always open whenever I ran into a trouble spot or had a question about my research or writing. She consistently allowed this paper to be my own work but steered me in the right direction whenever she thought I needed it. Another thankful thought to Professor Telmo Nunes who helped me with the data analysis.

Finally, I must express my profound gratitude to my mother for all the care and help throughout all these years of hard work and for having sacrificed herself in behalf of my education. My grandparents for having raised me as a child and for making me smile even in their sickness, to my uncles who have always supported me in every decision I made.

I am also gratefully indebted to all the doctors, nurses and colleagues at the Teaching Hospital of The Faculty of Veterinary Medicine where I took my internship and for having helped me grow and become a better doctor every day.

Resumo

Objetivo: Este estudo teve como objetivo determinar os efeitos do uso de metadona como fármaco único de pré-medicação cirúrgica, na pressão intraocular, tanto em cães, como em gatos, submetidos a procedimentos cirúrgicos eletivos e/ou exames de diagnóstico complementares que necessitassem de sedação prévia.

Métodos: O grupo em estudo incluiu cães e gatos submetidos a sedação. Os valores de pressão intraocular foram medidos antes da administração intravenosa de metadona, na dose de 0,2 mg kg^{-1}, e após 10 minutos (T10) e 20 minutos (T20) da mesma. As medições foram efetuadas por tonometria de ressalto (Icare ®, Helsínquia, Finlândia) com o animal posicionado em decúbito ventral, sem uso de colar isabelino e com a cabeça mantida numa posição relaxada ao nível do tórax. Os valores da variável pressão intraocular, obtidos nos três tempos, foram comparados utilizando o teste de análise de variância (ANOVA) para medidas repetidas, com o auxílio do software R® 3.3.3 software e na sua extensão R-Commander. As diferenças foram consideradas significativas quando $p< 0.05$.

Resultados: O grupo em estudo foi composto por 32 cães e 5 gatos com uma média de idades de 8,6 ± 3,3 e 6,02 ± 5,3 anos de idade, respetivamente. Os valores da média ± desvio padrão da pressão intraocular basais (T0) e após a administração de metadona (T10 e T20) foram, respetivamente: 17,1 ± 3,32 mm Hg, 16,9 ± 3,37 mm Hg e 16,3 ± 3,33 mm Hg. Na maioria dos indivíduos, os níveis de pressão intraocular diminuíram de forma menos marcada em T10 comparativamente com T20. Não se observou diferença estatisticamente significativa entre os três tempos em estudo ($p=0,296$).

Conclusões: Não se observaram variações estatisticamente significativas na pressão intraocular em cães e gatos após a administração de metadona como fármaco único de pré-medicação. Este fármaco pode ser uma boa opção na cirurgia intraocular ou na sedação de animais com diagnóstico prévio de glaucoma, uma vez que aparentemente não interfere com a pressão intraocular.

Palavras chave: glaucoma, pressão intraocular, opióides, metadona, cão, gato

Abstract

Objective: The purpose of this study was to determine the effects of methadone as a solo-agent of anaesthetic premedication, on intraocular pressure (IOP) in dogs and cats undergoing both elective surgeries or diagnostic procedures.

Methods: The study group was composed of 32 and 5 cats. The baseline IOP (T0) of the subjects were registered before IV methadone at the 0.2 mg kg^{-1} dosage. IOP variations were registered ten (T10) and twenty (T20) minutes after the drug administration. IOP values were measured with rebound tonometry (Icare ®, Helsinki, Finland), each animal being positioned in sternal recumbency, without e-collars and with the head maintained relaxed at the level of the thorax. All variables were compared at each specific time point using a repeated-measures analysis of variance (ANOVA) with R® 3.3.3 software and the R-Commander extension. The differences were considered significant when $p < 0.05$.

Results: The study group was composed of 32 dogs with a mean age of 8,6 ± 3,3 years and 5 cats with a mean age of 6,02 ± 5,3 years. Ophthalmic exam was normal. The mean ± SD baseline (T0) and post-treatment (T10, T20) IOP values were respectively: 17,1 ± 3,32 mm Hg, 16,9 ± 3,37 mm Hg and 16,3 ± 3,33 mm Hg. In the majority of the individuals, IOP levels decreased less significantly at T10 comparing to the mean values at T20. There were no statistically significant differences between baseline values and post-treatment values ($p=0.296$).

Conclusions: There were no statistically significant variations in IOP values in dogs and cats after the administration of methadone as a solo-agent of anaesthetic premedication. Methadone may be a good alternative as anaesthetic premedication in intraocular surgery or in sedation of glaucoma patients since it apparently does not interfere with IOP.

Keywords: glaucoma, intraocular pressure, opioids, methadone, dog, cat

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Nomenclature

Ach - Acetylcholine

AH – Aqueous humour

CAI - Carbonic Anhydrase Inhibitors

CB – Ciliary Body

CC – Ciliary cleft

ICA – Iridocorneal angle

IOP – Intraocular pressure

OCT – Optical coherence tomography

ONH – Optic nerve head

PGA - Prostaglandin analogues

PL – Pectinate ligament

PLD – Pectinate ligament dysplasia

POAG – Primary Open-Angle Glaucoma

PACG – Primary Angle-Closure Glaucoma

RGC – Retinal ganglion cells

RPE – Retinal pigmented epithelium

TM – Trabecular meshwork

UO – Uveoscleral outflow

Glossary

Blepharospasm: spasm of the orbicularis oculi muscle causing eyelid closure. Considered a sign of ocular pain.

Buphthalmia: pathologic enlargement of the globe due to chronically and notably elevated intraocular pressure (IOP); pathognomonic change in glaucoma. Inherited as an autosomal recessive trait in New Zealand white rabbits which are used as an animal model of glaucoma. Especially notable in young animals because of the relative distensibility of their sclera.

Epiphora: an abnormal overflow of tears down the face, due to excess production (secondary to painful ocular conditions) or reduced outflow through the lacrimal system.

Hyphema: red blood cells in the anterior chamber of the eye.

Hypopyon: white blood cells in the anterior chamber of the eye.

Mydriasis: dilatation of the pupil. May be physiologic, due to reduced parasympathetic and increased sympathetic tone to the iris sphincter and dilator muscles, or pathologic.

Miosis: constriction of the pupil. May be physiologic, due to increased parasympathetic and decreased sympathetic tone to the iridal sphincter and dilator muscles, or pathologic.

Photophobia: abnormal visual intolerance to light. Expressed in animals by excessive closing of the eyelids when exposed to light. However, since it is a symptom, it is impossible to differentiate from blepharospasm due to any painful eye disorder.

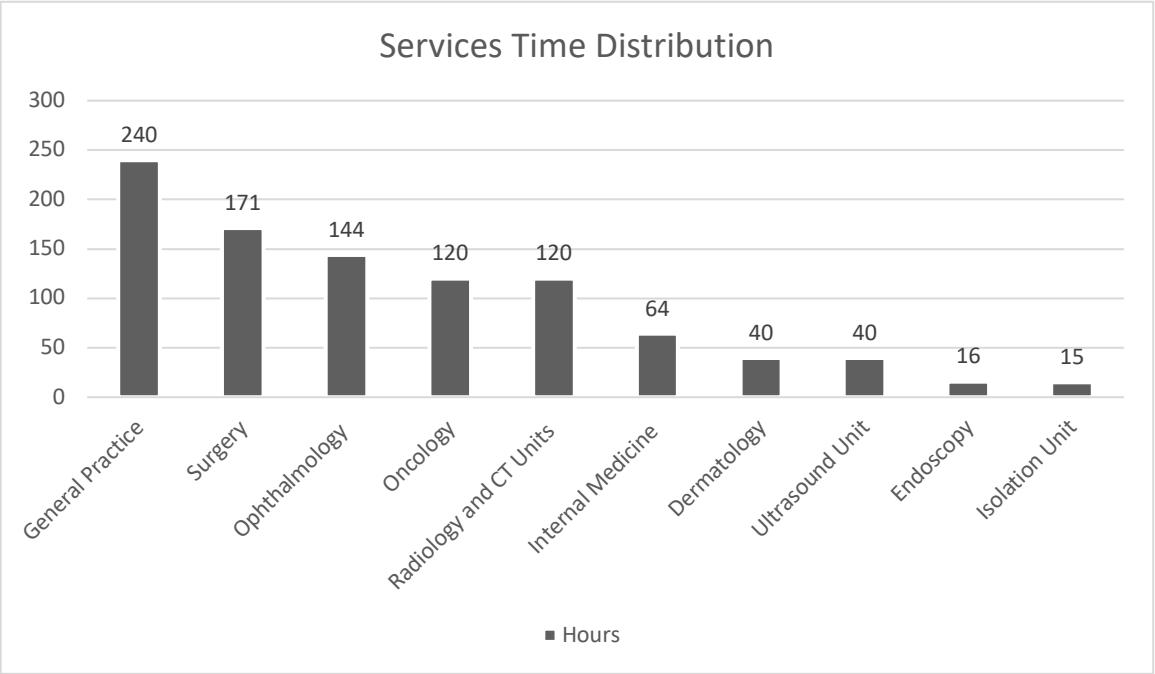
Synechia: adhesion of the iris to the cornea (anterior) or the lens (posterior).

Chapter I – Traineeship report

The internship at the Teaching Hospital of The Faculty of Veterinary Medicine, University of Lisbon, under the supervision of Dr. Esmeralda Delgado, began on March 5th and ended on August 31st, having the total duration of six months.

The rotation between several major departments provided countless opportunities to be in contact with a variety of clinical cases. The hospital runs a twenty-four hours permanent service with the clinical specialties of medical imaging (ultrasound, radiology and computed tomography scan units), surgery, endoscopy, internal medicine, ophthalmology, dermatology and oncology; and runs also a hospitalization and isolation units. The distribution time spent by the author in each speciality and unit is described in the graphic 1.

Graphic 1 - Absolute frequency distribution of the number of hours in each service department



The hospitalization shifts consisted of twelve hours during day or night shifts and the isolation unit held shifts of five hours each. The prescribed drugs in each patient chart were administrated by oral, intramuscular, subcutaneous or intravenous routes. Peripheral veins catheterization, collection of blood samples, glycemia measurements, blood arterial pressure monitoring and blood transfusions were some of the assignments. Nutritional support, palliative care and comfort of all the terminally ill patients was also provided.

At the surgery service, the student had the opportunity to calculate dosages, prepare and administer pre-anaesthetic drugs, place venous catheters for intravenous administrations and fluid therapy, prepare the anaesthesia induction, perform endotracheal tube placements and skin preparation of surgical sites. During surgery, the internee had the opportunity to help the main surgeon, be a circulating assistant or an anaesthetist assistant. As a surgeon's assistant, the internee would also help the lead surgeon in tissue retraction and skin closure.

During both general practice and speciality consultations, the reception of the animal, the collection of the medical history or anamnesis and the performance of the physical exam would also be conducted by the student. Blood collection samples, vaccine administration or other drug preparations and placement of intravenous catheters were also practiced during consultations. The discussion of the case in terms of differential diagnosis, complementary diagnostic exams, therapeutic plan, follow-up plan and prognosis would occur after the consultation with the respective doctor in charge. This process also allowed the development of the clinician-to-owner communication skills, a component that is crucial to clinical practice and which is often undervalued.

At the imaging service, the positioning of the animals, selection of x-ray screening constants, preparation of contrast agents and the contribution to the interpretation and discussion of the imaging results were also the major assignments.

Chapter II - Literature Review

1. Rationale for this study

The effects of anaesthetic agents on the ocular physiology should be thoroughly understood by the ophthalmic surgeon so that the regulation of IOP is done properly in order to not be greatly affected. A good anaesthetic management should minimize any potential side effect and avoid an increase in IOP over the entire anaesthetic period.

Sedation may be of extreme value as an adjunct to manual restraint to ease the handling of veterinary patients and also reduce the anxiety associated with ophthalmic examination. Ocular pain when severe enough to enable a thorough ophthalmic examination may also be minimized with a good analgesia control. For these reasons, the need for understanding the effects, in particularly of methadone, in intraocular pressure on behalf of animals suffering from glaucoma or animals undergoing intraocular surgery is of paramount importance.

The objective of the study reported here was to determine the effects of methadone on IOP both in dogs and cats, admitted to the Teaching Hospital of The Faculty of Veterinary Medicine, University of Lisbon, for surgical correction of ocular and extraocular diseases. The author hypothesized that methadone administration, independent of other preanesthetic medication, would not be associated with a significant increase in IOP in both dogs and cats.

For purposes of a better understanding of all the topics related to this subject, a brief summary of the anatomy of the eye and its outflow structures, glaucoma and drug effects on IOP, are fully explored in this literature review.

2. Anatomy of the Eye's Outflow Structures

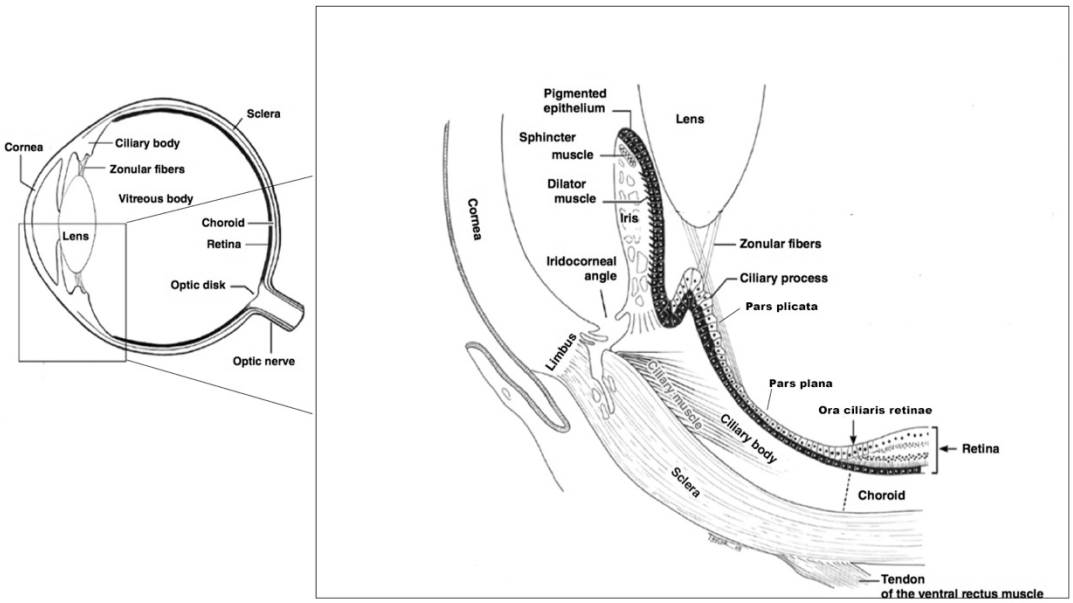
2.2. Brief review of the eye’s outflow structures anatomy

2.2.1. Ciliary body (CB)

Represents the anterior extension of the choroid joining with the iris (Samuelson, 2013). The CB is divided into an anterior segment - *pars plicata* – and into a posterior flat segment - *pars plana* (fig. 1). The *pars plicata* comprises the ciliary muscle, the ciliary vessels and the ciliary processes. Altogether these structures are responsible for the suspension and accommodation of the lens, the production and outflow of the

aqueous humour, the maintenance of the blood-aqueous barrier and the synthesis of acid mucopolysaccharide components of the vitreous (Trattler, 2012).

Figure 1 - Structural components of the eye in a sagittal section and emphasizing the topography of the cornea, iris, ciliary body and sclera. Adapted from Uemura, 2015.



The CB muscle (figure 1) is composed of smooth muscle in mammals (Gellat, 2013) and its fibers are primarily oriented meridionally in the dog. The CB muscle contracts under parasympathetic stimulation of the oculomotor nerve which also innervates the pupillary sphincter muscle of the iris (Jeruma, 2015). Adrenergic nerve endings are also present to a lesser extent, located in the subepithelial portions of the CB (Gellat, 2013).

The vitreal surface of the CB is covered by an inner nonpigmented epithelium and an outer pigmented epithelium (Watté, 2014). This double layer continues posteriorly with the sensory retina at the ora ciliaris retinae and anteriorly with the posterior PE of the iris (Ofri, 2013). The PE, facing the sclera, is continuous with the retinal PE (fig. 1). These two epithelial layers continue anteriorly as the bilayered iris epithelium (Ofri, 2013).

The CB processes can be described as finger-like projections composed of a pigmented epithelium and nonpigmented epithelium bilayer epithelial, capillaries and stroma (Trattler, 2012).

The iridocorneal angle (ICA) is formed from the anterior recession of the CB, the base of the iris and the corneoscleral tunic and is also known as the filtration angle or anterior

chamber angle. The width of the ICA can vary depending on breed and age within each specie (Pizzirani, 2015).

The lenticular zonules are fibers arising from the peaks of the CB processes and are attached along the epithelial surface in a criss-cross pattern on the lens capsule, anterior and posterior to the equator. As the CB muscle contracts and relaxes, the tension on those fibers decrease and increase, respectively, allowing the process of accommodation (Lowe, 2014). The accommodation process aims to maintain a clear image of both near and far objects. Therefore, the adjustment of the lens position and curvature can properly lead to the optimal focal length, converging the received light beams at a single spot in the retina. Making the comparison to human's level of accommodation, this capacity is known to be limited in dogs and cats (Watté, 2014).

Contraction of the CB muscle not only provides accommodation but also increases the conventional aqueous outflow (Watté, 2014) by moving the lens forward and increasing the lens curvature by releasing tension on the zonular fibres and lens equator.

CB vassels are represented by two long posterior ciliary arteries, vortex veins and anterior ciliary arteries arising from branches of the ophthalmic artery (Gellat, 2013; Mitchell, 2014).

2.2.2. Pectinate ligaments

The pectinate ligaments (PL) consists of beams of iris tissue that span the ICA and connect the base of the iris to the cornea. The junction between the cornea and the PL can be seen at gonioscopy and it is seen as a pigmented line known as the Schwalbe's line. These ligaments vary in number, pattern, length and thickness among different breeds and individuals and they are usually pigmented unless in sub-albinotic animals (Pizzirani, 2015).

2.2.3. Ciliary Cleft

The ciliary cleft (CC) represents the space posterior to the PL and juxtaposed to the sclera and filled with a sponge-like network of connective tissue beams, the trabecular meshwork (TM) (Watté, 2014; Hollingsworth, 2017). The TM and the CC are both contained within the ICA.

2.3. Aqueous composition, production and drainage

The aqueous humour (AH) corresponds to an optically clear fluid under regular conditions, which is produced by the CB and occupies both the anterior and the

posterior chambers. The cells responsible for its production are localized at the CB posterior nonpigmented epithelium, lining the ciliary processes.

Circulation of the AH has major purposes regarding the nourishment and the metabolic waste removal of avascular structures like the lens and the cornea; and it is of the upmost importance in maintaining physiological intraocular pressures (IOP), allowing an unaltered refractive status of the eye. In fact, the AH constitutes an important component of the eye's optical system as it provides a transparent and colourless medium of approximately 1.33 refractive index (Gabelt, 2011), between the cornea and the lens.

The balance between aqueous production and aqueous outflow maintains the IOP physiologic range which may vary individually in consequence of daily circadian patterns and aging (Pizzirani, 2015).

Both in humans and dogs, it was identified higher normal IOP values measured in the morning and lower values in the evening. On the contrary, in cats, rabbits and nonhuman primates, is has been suggested the opposite (Miller, 2013). It seems also unanimous that both production and outflow of AH tend to decline with age, being the rate of the former higher in the majority of animals and humans (Miller, 2013).

The production of AH is conducted by both passive and active processes. The passive mechanisms do not contribute significantly to the formation of AH although they are able to generate a fluid reservoir within the CB. On the opposite, the active mechanisms are responsible for at least 80-90% of the AH formation (Pizzirani, 2015).

2.3.1. Passive production mechanisms

The passive production mechanisms can be described as two distinct processes of diffusion and ultrafiltration of plasma, both taking place in the vascularized CB stroma. Diffusion represents the passive passage of AH along with lipid-soluble molecules in favour of their concentration gradient, across the fenestrated CB vassels endothelia (Pizirrani, 2015). The ultrafiltration process occurs in consequence of an uprising in the hydrostatic pressure which forces the passage of water and water-soluble molecules through fenestrations in the CB endothelium (Miller, 2013).

2.3.2. Active secretion

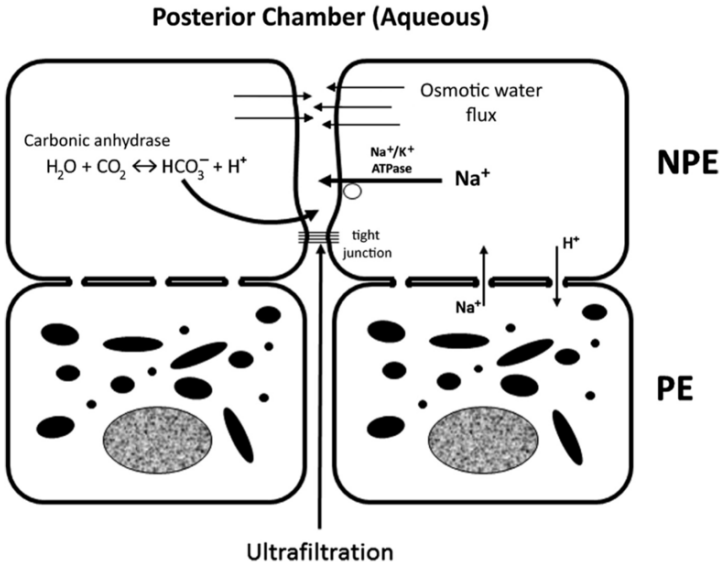
The majority of AH production is catalysed by carbonic anhydrases which transform carbon dioxide and water into carbonic acid by the equation 1.

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+$$

Equation 1 – Conversion of carbon dioxide and water into carbonic acid and its dissociation in bicarbonate and hydrogen ions.

The existence of sodium/potassium adenosine triphosphatase (ATPase) pumps (figure 2), that actively secrete sodium ions to the posterior chamber seem to drive negatively charged ions like bicarbonate. The entry of these solutes into the posterior chamber is accompanied by the diffusion of water, therefore composing the AH (Renwick, 2014).

Figure 2 - Mechanisms for AH secretion ATPase – adenosine triphosphatase; NPE – nonpigmented epithelium; PE – pigmented epithelium. Adapted from Pizzirani, 2015.



2.3.3. Trabecular Outflow (TO) or conventional/anterior outflow

TO is the major aqueous drainage system also known as the anterior or conventional outflow route. The AH passes from the posterior chamber, through the pupil, and into the anterior chamber (Miller, 2013). At this level, the AH is filtered through the TM achieving the angular aqueous plexus, composed of a series of collecting vessels that are anatomic and functional similar to the Schlemm's canal in primates (Pizzirani, 2015). The angular aqueous plexus eventually may drain the AH both anteriorly to the episcleral and conjunctival veins, or posteriorly into the scleral venous plexus and the choroidal vortex venous system (Miller, 2013).

2.3.4. Uveoscleral outflow (UO) or unconventional/posterior outflow

Occurs when the AH has entered the TM and escapes passively the TO through the CB muscle interstitium reaching the suprachoroidal space (Pizzirani, 2015).

3. Definition and Pathophysiology of Canine Glaucoma

During the decade of the 1950s, the term glaucoma was often referred to as a sign rather than a disease per se, and its definition comprised a group of diseases with an abnormal elevation of the IOP regardless of their nature, both in human and in veterinary medicine. Therefore, since 1980s, the plural form of the name, glaucomas, started to be adopted given the variety of factors that can influence the IOP in normal individuals (Gellat, 2014).

In humans, the most recent studies concluded that in addition to the two major forms of human glaucomas, Primary Open-Angle Glaucoma (POAG) and Primary Angle-Closure Glaucoma (PACG), there are some POAG individuals with normal IOP measurements and this exception created a new POAG category, referred as normotensive glaucoma. Although normotensive glaucoma does not meet the 21-mmHg cut-off criterion for 'elevated IOP' in humans, IOP is probably still too high in these eyes (Tan & Kaufmann, 2014).

In all of the human glaucomas forms, including normotensive glaucoma, the progressive optic neuropathy with visual field loss, the thinning of the retinal nerve fibre layer and the excavation of the optic nerve head are present (Saeedi, Ramulu & Friedman, 2014). For this reason, the IOP increase which has been historically a common definition for the disease in small animals, is not mentioned in the accepted definition used by physician ophthalmologists. The fact that even a controlled normalized IOP does not alter the progression of the disease in both humans and dogs, suggests that additional pathophysiologic mechanisms must be involved (Pizzirani, 2015).

Some of the risk factors leading to the development of some glaucomas forms are:

a. Constant IOP raise

The reduction in choroidal vascular perfusion impairs both microcirculation and the axoplasmic flow in the retinal ganglion cell axons leading to outer retinal necrosis (Martín, 2017).

b. Age

Age-related abnormalities within the TM may increase the outflow resistance. These changes can include the thickening of basal and trabecular membranes, partial loss of endothelial cells and the accumulation of materials such as pigment granules and collagen (Pizzirani, 2015).

c. Familiar history

The genetic inheritance seems to be an important risk factor both in humans and dogs with some causative genes being already identified (Komaromy, 2015).

d. Breed and presence of goniodysgenesis

Goniodysgenesis corresponds to a defect in the development of the ICA which has its width decreased due to the adjacent pectinate ligaments dysplasia (PLD). These ligaments undergo a process of consolidation, forming a broad sheet structure which may be excessively extensive but can also, in some cases, be perforated by intermittent flow holes (Renwick, 2014). It is believed that PLD has genetic causes considering the high incidence in certain breeds (Pizzirani, 2015) but it has not been directly related to an increased resistance to the outflow unless the condition progresses and affects over 180º of the ICA (Gellat, 2014). It is also important to differentiate PLD from inflammatory associated anterior synechiae (Gellat, 2014).

e. Systemic blood hypotension

Systemic hypotension can dysregulate ocular perfusion by primary tissue ischemia and is sufficient to induce permanent damage in RGC if hypoxia is superior to a six hours duration period (Pizzirani, 2015).

Additional factors that can raise IOP in healthy animals:

- **Positioning and degree of restraint**: any increase in venous pressure reduces venous drainage and AH outflow from the eye, increasing the IOP (Clark, 2014). The use of collars, neck leads or excessive restraint may cause this jugular occlusion.
- **Pressure on the ocular globe or forced lid opening**: exophthalmic or brachycephalic breeds are more prone to this occurrence (Renwick, 2014)
- **Diurnal variation**: in dogs and humans, the IOP is slightly increased in the morning and become gradually normal during the day (Miller, 2013).

- It is also undeniable that other factors including anatomy variations of the anterior segment and endogenous compounds such as neurotransmitters, hormones, prostaglandins among many others, can influence these mechanisms and alter the IOP baseline (Miller, 2013).

3.2. Classification of canine glaucomas

Canine glaucomas can be classified according to their aetiology, the gonioscopic appearance of the filtration angle (ICA and CC) and the staging of the course of the disease (table 1).

Table 1 – Aetiology of canine glaucomas. Adapted from Gellat, 2014.

- Aetiology
    - Primary
        - Open ICA / normal CC
        - Narrow ICA /closed CC (the majority caused by goniodysgenesis)
        - Both can be acute or chronic
    - Secondary
        - Uveitis
        - Lens luxations
        - Intumescent cataract
        - Phakolytic/phacoclastic uveitis
        - Hyphema
        - Intraocular neoplasia
        - Aphakic
        - Malignant/ciliary block
        - Melanocytic/Pigment cell proliferation
        - Pigment cell exfoliation/anterior uveal cysts
        - Giant retinal tears (Schwartz-Matsuno syndrome)
        - Anterior chamber silicone oil
        - Postoperative ocular hypertension
    - Congenital
        - Pectinate ligament dysplasia/Goniodysgenesis
- Iridocorneal angle (ICA)
    - Open / Narrow / Closed

- Ciliary cleft (CC)
    - Open / Narrow / Collapsed
- Staging
    - Acute/ Chronic

3.2.1. Congenital Glaucoma

This severe and rare form of glaucoma develops due to an abnormal formation of the anterior segment including the TM, immediately after birth or within the first six months of life (Martin, 2017). The first clinical signs consist of rapid onset of buphthalmia, inability to close the palpebral fissure and development of exposure corneal disease (Gellat, 2014).

3.2.2. Primary Glaucoma

Primary glaucomas are defined as a group of disorders in which occur characteristic changes to the optic nerve without any proven association with other ocular or systemic disorder (Miller & Bentley, 2015). They emerge in approximately 20% of the total dog glaucomas and they are thought to have a genetic inherited aetiology, associated with breed predisposition and present themselves as bilateral even though they can be asymmetrical (Martín, 2017). They can be classified according to the gonioscopy appearance of the drainage angle in POAG and PCAG.

3.2.2.1. **Primary Open-Angle Glaucoma (POAG)**

The drainage ICA appears normal and indistinguishable from a healthy eye. Recent evidences of an increase in altered glycosaminoglycans (GAGs) forming the TM and ultimately leading to a deposition of elastin membranes and type IV collagen are considered to contribute to the resistance of AH flow, causing IOP to rise (Martín, 2017). An enzyme defect allowing the accumulation of extracellular matrix material in the TM is also questioned (Miller, 2013). This type of glaucoma accounts for about 3% of the total dog glaucomas (Martín, 2017).

3.2.2.2. **Primary Angle-Closure Glaucoma (PACG)**

PACG is the most common form of canine primary glaucoma and is a complex trait with multiple genetic and possibly environmental risk factors (Komáromy, 2015). According to Martín, 2017, approximately 80% of the PACG's in dogs are due to PLD, an abnormality already explained in page 8. Besides the majority of goniodysgenesis-

related glaucoma, anomalous conformation of both a narrow CC and anterior chamber, anterior displacement of the lens and iris plateau may also contribute to PACG (Martín, 2017). The iris plateau abnormality has been described as an anatomic variant of the iris structure in which the iris periphery angulates sharply forward from its insertion point and then again angulates sharply and centrally backward (Diniz Filho, 2008). PACG represents itself as a bilateral disorder although it might be initially unilateral. The raise in IOPs is marked and rapid, affecting middle-aged to older dogs of certain breeds (see table 2) (Miller, 2013).

Female dogs can possibly have a shorter axial globe length and a narrower ICA opening and for these reasons they are twice as likely to be affected as male dogs (Komáromy, 2015).

Table 2 – Glaucoma commonly affected breeds of dogs. Adapted from Martín, 2017.

Commonly Affected Breeds of Dogs

POAG	PCAG	Goniodysgenesis
American Cocker Spaniel	American Cocker Spaniel	French Bulldog
Basset Hound	Akita Inu	Basset Hound
Boston Terrier	Samoyed	Great Dane
Miniature schnauzer	Beagle	Samoyedo
Beagle	Siberian Husky	Chow-chow
Chow chow	Labrador Retriever	
Siberian Husky	Toy poodle	
Standard poodle		

3.2.3. Secondary Glaucomas

Secondary glaucomas are common both in dogs and cats but particularly on the latter where 95 to 98% of the feline glaucomas can be categorized as secondary (Pumphrey, 2015).

3.2.3.1. Uveitis

Responsible for up to 45% of secondary glaucomas in dogs and both uveitis and glaucoma may occur at the same time or glaucoma can develop months to years after the uveitis episode (Pumphrey, 2015). The mechanisms in which the inflammation of

the uveal tract induces glaucoma are predominantly linked to the deposition of inflammatory debris, such as blood components and fibrin, obstructing the pupil, ICA or TM (Pumphrey, 2015; Renwick, 2014). Those deposits can form adhesions between the iris and the lens (posterior synechiae) preventing AH flow through the pupil and being withheld behind the iris which bulges forward. The formation of what is known as “iris bombé” occurs when these adhesions are present at 360 degrees. Peripheral anterior synechiae can also develop associated with iris bombé, luxation of the lens by pushing the iris forward or intumescent cataracts (Renwick, 2014)

The CC is also composed of uveal tissue and any swelling of the TM structures can therefore cause an additional block of the AH outflow.

Uveitis and glaucoma should be well differentiated by tonometry because they may both be presented as a red and painful eye (Miller, 2013).

3.2.3.2. Cataracts and cataract surgery

The leakage of antigenic lens proteins into the AH is induced in mature or hypermature cataracts associated with lens rupture, inducing a secondary glaucoma by the inflammation it creates (Renwick, 2014). This lens induced uveitis aetiology is not clear but the presence of keratic precipitates and inflammatory cell clumps are often present (Pumphrey, 2015).

3.2.3.3. Lens instability and lens luxation

Lens luxation can occur primary or secondary to a glaucoma. The primary lens luxation (PLL) or subluxation can induce glaucoma, especially if the luxated lens enters the anterior chamber causing a pupillary block and impairing the AH outflow (Miller, 2013). In the second scenario, the primary glaucoma in a buphthalmic globe, stretches and subsequent tears off the zonular fibres which support the lens, resulting of its subluxation or even complete luxation (Miller, 2013).

3.2.3.4. Neoplasia

There are multiple ways in which an intraocular neoplasia can induce glaucoma. The direct mass effect or the accumulation of exfoliated neoplastic in the ICA or TM may impair normal aqueous drainage (Pumphrey, 2015).

Primary uveal melanoma, primary ciliary body adenoma/adenocarcinoma and lymphoma are the most common types of neoplasms (Renwick, 2014). Metastatic

neoplasms account for about 4% of the total ocular neoplasia in dogs and the involvement may be bilateral (Pumphrey, 2015)

In the Comparative Ocular Pathology Laboratory of Wisconsin (COPLOW) collection, of the 5722 cases of neoplasia, 1516 had glaucoma as part of the syndrome and with the following definitive histopathological type: 57% melanoma, 21% iridociliary epithelial tumours, 5% metastatic tumours and 4.5% lymphoma.

The pathogenesis of PACG due to neoplasms can be explained by the mechanical displacement of the iris and lens by the mass effect in neoplasms of the CB, choroid, or retina, which leads to pupillary blockage (Dubielzig, 2010).

3.2.3.5. Hyphema

The presence of blood in the anterior chamber, which may also appear along with hypopyon or fibrin, can occlude the pupil, the ICA or the TM by the deposition of blood component cells and debris (Pumphrey, 2015). Lower IOP in cases of trauma and severe intraocular haemorrhage may be present due to CB damage or scleral rupture leading to a marked decrease in aqueous production (Renwick, 2014).

3.3. Pathophysiology

The retina is embryologically derived from the optic cup, which is a double layer of neural ectoderm, and it is divided in a neurosensory or inner layer which is non-pigmented and in the retinal pigmented epithelium (RPE), which forms the outer layer (McLellan, 2014).

The optic nerve head (ONH), also known as the optic disc or optic papilla, is the beginning of the myelination of the retinal ganglion cell (RGC) axons as they converge to exit the globe through the lamina cribosa of the sclera. The continuous mechanical compression constricts and obstructs the axoplasmatic flow and allows the apoptosis of the nerve cells to initiate. The impairment of the physiological movement of molecules impairs the transport of neurotrophins, which are essential for the development and maintenance of the neurons function and promotes the accumulation of oxygen free radicals and excitotoxins like glutamate (Martín, 2017). Glutamate in high concentrations induces the entrance of sodium, chloride and water into the cells causing oedema and cell membrane rupture (Pizzirani, 2015).

The high levels of calcium outside the axons seems also to generate more free radicals which activates in their turn, proteases, kinases and phospholipases. All of these

events lead to an optic neuropathy in which the RGC undergo apoptosis and an irreversible blindness occurs (Martín, 2017; Pizzirani, 2015).

3.4. Clinical signs

Clinical signs of glaucoma are directly related to the level and duration of the elevation in the IOP (Gellat, 2014) and are similar regardless of the cause of the elevation (Miller, 2013). Most owners will not be able to recognize the disease early in its course until the IOP approaches 40 mmHg or until there is noticeable loss of vision (Martín, 2018).

3.5. Early to mid-stage glaucoma clinical signs

3.5.1. Pain

In general, animals tend to disguise evident signs of pain, therefore a good anamnesis should be accurately obtained. Complaints of lack of interest in playing or climbing on the couch, starting to hide in unusual locations, having reduced tolerance for disturbances, having loss of appetite or spending too much time sleeping can be the first indicators of lethargy and pain. On the other hand, other animals can exhibit continuous blepharospasm, epiphora, photophobia and protrusion of the nictitating membrane (Martín, 2017), particularly when the IOP is above 40-50 mmHg where the discomfort may be so severe as to cause vocalization (Renwick, 2014). Blepharospasm can also be present in both eyes even when only one is affected. This fact might be explained due to a generalized migraine sensation, in similarity with what is reported in human glaucomas (Miller & Bentley, 2015).

3.5.2. Pupil alterations

Mydriasis, the dilation of the pupil is often present in acute glaucomas with IOPs above 40-50 mmHg, causing pressure high enough to induce paresis or paralysis of the iris sphincter muscle (Martín, 2017). If glaucoma is associated with uveitis in the same eye, the pupil may be normal or even constricted (Martín, 2017; Renwick, 2014). The direct pupillary light reflex is reduced or absent which does not equal a total loss of vision. In this situation, the potential vision must rather be assessed with the testing of the menace response, dazzle reflex or the consensual pupillaru light reflex in the unaffected eye, illuminating only the affected eye (Renwick, 2014).

Peripheral anterior synechia can be formed rapidly and the ischemia originated also in the iris sphincter muscle can be responsible for sectorial or diffuse atrophy of the iris (Miller & Bentley, 2015).

3.5.3. Corneal Oedema

The normal optical clarity of the cornea depends primarily of its state of dehydration. This state is achieved owing to the active transport mechanism of the cornea endothelium, which transports solutes from the cornea into the aqueous humour, against the IOP and causing the movement of water out of the corneal stroma by diffusion (Renwick, 2014; Maggs, 2013). Although the increase in IOP alone is not sufficient to disrupt this mechanism, persistently elevated IOP drives fluid across the corneal endothelium, creating oedema of both epithelium and stroma. (Miller & Bentley, 2015). In conclusion, corneal oedema results when excess fluid accumulates within the stroma and forces the collagen lamellae apart, leading to loss of transparency (Maggs, 2013).

The ability to blink and distribute the tear film can be compromised with the globe enlargement which can result in the formation of irregular areas in the epithelium or even ulceration mainly in the centre of the cornea (Renwick, 2014).

3.5.4. Episcleral congestion

The increase in IOP reduces the flow through the CB to the vortex veins leading to the flow to pass via anastomosis through the episcleral veins and capillaries to a lesser extent (Miller, 2013). The engorgement of superficial conjunctival vessels indicates an ocular surface disease and for this reason it is important to be able to distinguish them from the episcleral vascular vessels with the features presented at table 3.

Intermittent episcleral injection can sometimes be the only early sign in patients whose IOP remains normal on the physical exam (Miller, 2013).

Table 3 - Morphological and clinical differences between episcleral and conjunctival vessels

Episcleral vessels	Conjunctival vessels
• Dark red • Larger calibre and run at right angles to the limbus • Do not move in association with movement of the overlying lids and conjunctiva • Blanch themselves poorly and slowly after the application of topical 1% adrenaline or 10% phenylephrine	• Bright pink to red • Cover larger portions of the sclera • Typically branched • Blanch themselves after the application of topical 1% adrenaline or 10% phenylephrine within 1 or 2 minutes

Episcleral congestion can also be present, apart from glaucoma in cases of:

- Uveitis / endophthalmitis / panophthalmitis
- Episcleritis (often localized in dogs)
- Retrobulbar space-occupying lesion (e.g. abscesses, neoplasia)
- Horner's syndrome
- Hyperviscosity syndromes
- Excitement

3.6. Chronic glaucoma clinical signs

Chronic glaucoma can develop after an uncontrolled or misdiagnosed of an acute-onset episode or it may progress from an insidious first episode. In chronic cases there also may be present some of the clinical signs seen in acute glaucomas although less markedly. The following chronic related signs indicate poor prognostic for vision.

3.6.1. Buphthalmia / Buphthalmos

Pathologic enlargement of the ocular globe due to chronically and notably elevated intraocular pressure and a pathognomonic change in glaucoma. It is often responsible for irreversible blindness (Martín, 2017), although limited vision may be retained for a while in puppies and sharpeis (Miller, 2013). The differential diagnosis with exophthalmos must be made, as this sign occurs in a normal sized eye that is being pushed forward by a retrobulbar abscess or neoplasia that is occupying space (Renwick, 2014). This condition leads often to exposure keratitis, corneal ulceration, subluxation of the lens and occasionally scleral ectasia (Miller & Bentley, 2015). Lens

subluxation must also be carefully differentiated from PLL which also leads to secondary glaucoma (Miller & Bentley, 2015).

3.6.2. Luxation or subluxation of the lens

The fact that the lens starts to occupy a position away from the visual axis develops an aphakic crescent which becomes visible (Renwick, 2014). Movements of both the lens (phacodonesis) or the iris (iridodonesis) may also be seen, as well as an abnormally shallow or deep anterior chamber (Martín, 2017; Miller, 2013).
Lens opacities can also develop in chronic glaucomas due to poor lens nutrition and/or the accumulation of toxic products such as glutamate (Renwick, 2014).

3.6.3. Haab Striae / Descemet's Streaks

The pressure induced by the stretching of the globe can cause breaks in the Descemet membrane which can be detected as blue-grey streaks across the cornea (Miller & Bentley, 2015). Other grey opacities within the cornea may develop in cases of scar tissue formation following ulceration or secondary to exposure and subsequent keratinization of the cornea epithelium (Renwick, 2014).

3.6.4. Equatorial staphyloma

The stretching of the sclera can lead to its thinning at the equator of the globe and the formation of staphylomas (Renwick, 2014). This scleral defect is filled directly by uveal tissue and, after a few hours, by blood clots and exudate (Stades et al, 2007).

3.6.5. Phthisis Bulbi

Atrophy of the eye that may occur in advanced cases of glaucoma, when the CB no longer produces AH. This condition can also be a sequela of some therapies aimed at destroying AH production or to a lesser degree after gonio-implantation with excessive filtration (Miller & Bentley, 2015). When *phthisis bulbi* is present at the time of examination, the IOP values are typically normal or even reduced (Renwick, 2014).

3.6.6. Optic nerve atrophy and Retinal Degeneration

The optic disc represents the location where the optic nerve enters the ocular globe and all the retinal nerve fibres converge. The optic nerve axons are supported in a fenestrated scaffold within approximately a third of the inner sclera called lamina cribosa (Dawson, 2011). The sustained high IOPs and its mechanical compression of

the lamina cribosa connective tissues causes the cupping of the optic disc and compromises both the axonal flow and the blood supply, leading ultimately to the optic nerve axonal death (Plummer, 2013). The optic disc is presented with signs of cupping when it bows posteriorly through the lamina cribrosa (Miller, 2013).
The retinal thinning and atrophy follows the induced pressure of the choroidal vasculature which supplies the outer layers of the retina (Renwick, 2014). This results in a tapetal hyper-reflexion with an increased reflection of light back from the tapetum (lay within the choroid).

Some clinical findings are more prone to be observed in acute cases while others are more prevalent in chronic glaucoma cases as seen in table 4.

Table 4 – Different clinical aspects of acute and chronic glaucomas. Adapted from Martín, 2017

	Acute Glaucoma	**Chronic Glaucoma**
IOP	>30 mmHg	High, normal or low
Episceral congestion	Present	Present
Diffuse corneal oedema	Present	May be present. Haab striae. Exposure keratitis
Ocular globe size	Normal	Normal or buftalmic
Pupil	Midriasis	Midriasis or normal
Lens position	Normal	Normal, subluxated or luxated. Cataracts
Optic nerve	Normal	Atrophy
Retina	Normal	Vascular attenuation Tapetal hyper-reflectivity

3.7. Diagnosis

In addition to the clinical signals described, the first diagnostic methods that are fundamental to confirm the clinician suspicion can both be used to evaluate intraocular pressure or/and the ICA.

3.7.1. Tonometry

The measurement of IOP is of the utmost importance in diagnosis and monitoring ocular conditions in which it is predicted a disturbance of IOP regulation. As already mentioned, IOP above 25 mmHg is suspicious for glaucoma in dogs, and above 30 mmHg in cats (Martín, 2017).

The direct form of measuring IOP can be performed through paracentesis of the anterior chamber which due to its invasiveness it is highly impractical in clinical practice (Spiessen et al, 2015).

All of the currently available tonometers do not actually measure IOP directly but a physical property of the cornea and use it to estimate true IOP (Miller & Bentley, 2015).

- **Indentation** tonometry – the Schiotz tonometer measures the degree of corneal deformity by applying a standard force with a metal rod in a topically anesthetized cornea (Heinrich, 2014). The greater the distance of the rod indents into the cornea, the lower is the patient's IOP (Maggs, 2013). The cornea must be in a horizontal position and without excessive pressure on the neck, to obtain readings. This technique is the most difficult to use correctly, having the largest number of potential errors and its use is limited in patients with severely ulcerated corneas or with other pathologic defects (Miller, 2015; Heinrich, 2014).

- **Applanation** tonometry: the indirect IOP measured with these tonometers (e.g. Tono-Pen XL®, Tono-Pen Vet® or Avia®) results from the force required to flatten the corneal surface. The readings with these devices can be altered with corneal drying (after application of a topical anaesthetic) or increased tear film viscosity (from mucoid ocular discharge or artificial tears) (Miller & Bentley, 2015).

- **Rebound** tonometry: the Tonovet® and iCare® devices create a magnetic field to eject a small probe at a fixed distance from the cornea and after it reaches

the cornea surface it returns or rebounds to the instrument. The instrument assesses the probe deceleration (rebound) which allows the conclusion that eyes with a higher IOP cause a more rapid deceleration of the probe and shorter return time to the instrument than those with a lower IOP (Gellat, 2013). An altered ocular surface tension as seen with the application of topical medications (including topical anaesthetic) can affect the results with rebound tonometry. For this reason, this technique should be performed before the application of such medications (Maggs, 2013).

With all the methods described, it is extremely important to perform a careful restraint of the patient to prevent an IOP overestimation, avoiding an excessive compression both of the neck where the jugular veins are located and the ocular globe (Martín, 2017).

It is also advisable to obtain more than one measurement and if possible at different periods of the day.

3.7.2. Gonioscopy

Although the gonioscopy evaluation requires considerable practice, the technique allows the clinician to estimate the severity of the obstruction of the drainage angle, identifying the type of glaucoma present and the likely location of the impediment to the outflow. Since this procedure compresses the cornea it should never be performed before tonometry evaluation (Miller & Bentley, 2015). To perform gonioscopy, a goniolens is required (Barkan, Koeppe, Goldmann) and its application should be made upon the anaesthetised cornea without compressing it excessively (Martín, 2017). This technique may be useful in the diagnosis of abnormalities such as PLD or closure of the CC and pectinate ligament, infiltration by neoplastic tissue, inflammatory deposits or even abnormal pigment deposition and fibrovascular tissue ingrowth (Renwick, 2014).

A grading system is used to compare objectively not only eyes within the same patient and between different patients but also the same eye in distinct moments of assessment. The Shaffer scale modified by Ekesten and Narfstrom scores the width of the iridocorneal angle in the grades of 0 (closed), 1 (narrowed), 2 (slightly narrowed), 3 (open) and 4 (wide open) (Martín, 2017).

The European College of Veterinary Ophthalmologists (ECVO) adopted other nomenclature system based on the PLD occlusion, assuming that it is always

associated with an open ICA, an assumption that is not widely accepted (Pizzirani, 2015). In this system the grade of occlusion is graded ascending from A (slight occlusion) to C (almost complete occlusion).

3.7.3. Ophthalmoscopy

The cupping of the optic disc can be accessed by direct or indirect ophthalmoscopy but early detection of optic disc changes is extremely difficult because of the large amount of myelin over the disc surface in dogs (Miller & Bentley, 2015).

In cats, the optic disc cupping can be difficult to identify because of the physiological lack of myelin in their ONH, therefore a dark to pale grey appearance, peri-pigmentation around the ONH, a hyperreflective halo and the fact that blood vessels can no longer be observed crossing the rim of the ONH may cause suspicion (McLellan, 2015).

Other advanced techniques can give the clinician a more detailed description of anatomical structures, as to mention optical coherence tomography (OCT) and high frequency ultrasounds (Martín, 2017).

High frequency ultrasounds are only capable of studying the anterior segment of the eye due to its poor penetration strength but do it with extreme accuracy, all the anatomic details of the ICA aperture evaluating the cornea, sclera, CB and iris can be accessed, having a particular utility for the diagnose of primary glaucomas or intraocular neoplasia (Martín, 2017).

The OCT technique uses near-infrared light that allows the acquisition of cross-sectional sets and 3D images, approaching it to in vivo “histopathology” with distinct layers visible as alternating bright and dark signals (Miller & Bentley, 2015). The anterior segment structures can be detailed without the need of direct contact between the transducer and the surface of the eye, minimizing tissue distortion and trauma (Dennis, Johnson & McLellan, 2014).

3.7.4. Genetic Testing

As already reported, primary glaucoma, both POAG and PACG, have an important genetic predisposition although the studies within each breed are relatively scarce.

The studies conducted in POAG dogs reveal that they can be related to mutations in the genes ADAMTS10, ADAMTS17 and Fibrillin-1 (FBN1) causing modifications in the soft tissue composition and leading to an impaired microfibrils formation (Martín, 2017).

The ADAMTS10 codifies a type of metalloprotein expressed in the tissues that compose the TM and the mutation in this gene was identified in Beagles with POAG. Although far from being completely understood, it seems that the inheritance of this trace is recessive in Beagles (Komaromy, 2015). The ADAMTS17 mutation were associated with PLL in the Jack Russell Terrier, Bull Terrier and Lancashire Heelers, therefore, inducing a secondary glaucoma. Ultimately, the Fibrillin-1 is an important component of the lenticular zonules and is secreted by the NPE of the CB (Martín, 2017).

3.8. Differential Diagnosis

Although the IOP remains the primary clinical sign that distinguishes glaucoma, confusion can be made with the diagnosis of other ocular pathologies mainly in cases of uveitis, conjunctivitis and episcleritis. The following table helps to clarify the diagnosis process.

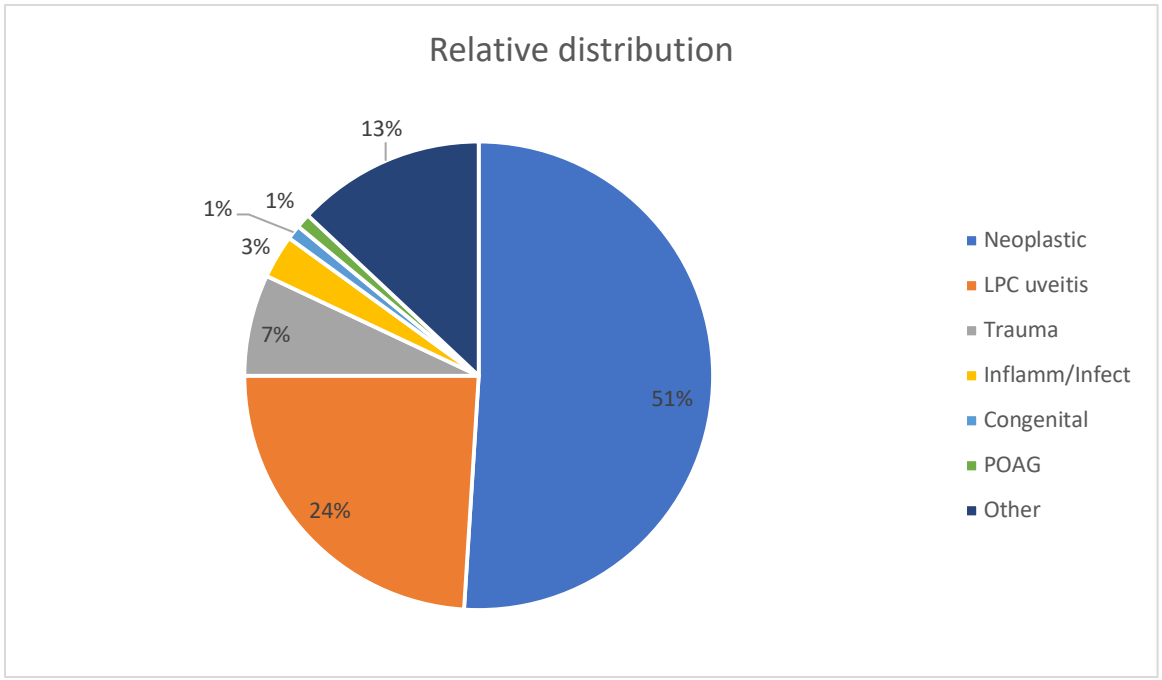
Table 5 – Differential diagnosis between acute uveitis, acute glaucoma, conjuntivitis and episcleritis. Adapted from Martín, 2017.

Clinical sign	Acute uveitis	Acute glaucoma	Conjuntivitis	Episcleritis
IOP	Normal / ↓	↑	Normal	Normal
Blepharospasm	√	√	√	√
Epiphora	√	√	√	-
Chemosis	-	-	√	-
Corneal Oedema	√	√	-	√ / Normal
Mydriasis	-	√	-	-
Miosis	√	-	-	-
Episcleral congestion	-	√	-	√
Iris rubeosis	√	-	-	-
Tyndall Effect	√	-	-	-
Hypopion	√/-	-	-	-
Hyphema	√/-	-	-	-

4. Feline Glaucomas

The majority of feline glaucomas are secondary to other ocular or systemic disease, primary glaucomas are rare (McLellan, 2015). According to the COPLOW, 29% of the 3212 feline enucleated globes entries were due to glaucoma, from 1983 to 2008 (Dubielzig, 2010). The relative distribution of the causes of feline glaucoma, identified by COPLOW follows in the graphic 2.

Graphic 2 - Relative distribution of feline glaucoma causes (Dubielzig, 2010)



4.1. Primary Glaucoma

Compared with dogs, goniodysgenesis appears to be quite rare in cats although PLD has been reported in Burmese cats (Renwick, 2014). POAG affects usually middle-aged to older adult cats (mean age around 10 years), unilaterally or bilaterally but most frequently asymmetric (McLellan, 2015). Inherited POAG is described in Siamese cats but PACG is extremely rare to non-existent (Miller, 2013).

4.2. Secondary Glaucoma

The majority of the total of glaucoma cases are due to neoplasia and uveitis. Diffuse iris melanoma and uveal lymphoma are the most common tumours (Renwick, 2014).

Exudative uveitis in cases of feline infectious peritonitis (FIP), toxoplasmosis, feline immunodeficiency virus (FIV) or feline leukaemia virus (FeLV) can also obstruct the CC by deposition of the lymphocytic / plasmocytic cellular component (Martín, 2017).
Other causes such as intraocular haemorrhage and hyphema due to systemic blood hypertension or trauma can also lead to glaucoma.
Primary lens luxation seems to have a low incidence in comparison with dogs and is usually associated with the underlying uveitis (Renwick, 2014).
In older cats, glaucoma may develop due to a shallow anterior chamber where there is an anterior displacement of the iris and lens by an expanded vitreous, creating a posterior misdirection of the AH rather than anteriorly (McLellan, 2015).

4.3. Clinical Signs

Cats often present clinical signs that are less severe because of the slow and insidious rise in IOP that tends to mask ocular pain although buphthalmia can be quite extreme (McLellan, 2015). Mydriasis, exposure keratitis and retinal degeneration are the most common signs and the loss of vision is slower than in dogs (Miller, 2013). In cases of uveitis, the signs are also very mild and a slit-lamp biomicroscopy should be performed to rule it out (Renwick, 2014).
A subtle anisocoria may be present especially in the eyes suffering from AH misdirection syndrome (Miller, 2013)

5. Medical Treatment of Glaucoma

The main criteria concerning the medical treatment of glaucoma is preserving the optic nerve and the RGC function for as long as possible, keeping the patient visual and without pain. Reducing IOP by enhancing the AH drainage or inhibiting its production, remains the mainstay of the medical management (Alario, 2015). The prophylactic use of neuroprotective and neurotrophic agents is also an important keystone.
The animal presented with a new diagnose of glaucoma should be first assessed in terms of the acuteness or chronicity of the disease or, in other words, if the eye still has the potential for vision or if it is irreversibly blind (Miller, 2013).
Acute glaucoma with more than 40-50 mmHg of IOP is usually associated with a very recent onset that requires emergency treatment (Martín, 2017).
The long-term control of glaucoma is usually not attainable regarding only medical therapy. The urgency of combined medical and surgical therapies is often required,

since the persistence of high IOP during 24 to 72 hours results in irreversible vision loss (Miller, 2013).

The majority of studies were conducted in population of research beagles affected with normotensive or POAG and these do not represent the typical PACG type of glaucoma that represents the vast majority of canine glaucomas (Alario, 2015).

5.1. Topical ocular hypotensive drugs

Topical hypotensive drugs are largely more effective than systemic therapy and with lower side effects. The major classes commonly used are presented below:

5.1.1. Cholinergic agonists or Miotics (Pilocarpine, Demecarium bromide)

The stimulation of acetylcholine (Ach) receptors of the parasympathetic nervous system in the eye leads to the CB muscle contraction and miosis, widening the conventional outflow pathway via the trabecular meshwork. Their major indication is for POAG since when goniodysgenesis happens or there is a CC collapse these agents will not prove effective (Renwick, 2014).

The direct-acting agents (pilocarpine and carbachol) activates the Ach receptors directly while indirect-acting agents (demecarium bromide) acts as an anticholinesterase increasing the concentration and time exposure of Ach to its receptors (Alario, 2015).

The miosis induced may lead to the formation of posterior synechiae and even iris bombé in cases where concomitant uveitis is present (Alario, 2015).

5.1.2. Drugs Acting on adrenoceptors

5.1.2.1. Nonspecific adrenergic agonists (Epinephrine and Dipivefrin)

Although not completely understood, these agents seem to decrease AH production due to decreased blood flow to the CB and they also seem to promote the conventional outflow by binding to β-receptors in the TM (Alario, 2015). These agents are not commonly used because of the strong mydriasis they promote (Renwick, 2014).

5.1.2.2. α_2-Adrenergic Agonists (Apraclonidine, Brimonidine)

The activation of α_2 receptors inhibits norepinephrine release and therefore the sympathetic stimulation of AH production at the CB epithelium is blocked (Alario, 2015). The use of apraclonidine in canine patients can cause inconsistent effects but

mainly bradycardia that ought to be monitored for about one-hour post administration (Renwick, 2014). Brimonidine seems to have little effect in canine patients (Alario, 2015).

5.1.2.3. β-Adrenergic Antagonists (β-Blockers) (Timolol, Betaxolol)

β-Blockers reduce AH production firstly by blocking β_2-receptors on the CB epithelium which also inhibits norepinephrine stimulation and secondly because of the block of sodium potassium ATPase which are responsible for the active transport and ultrafiltration process (Alario, 2015). Their effect is also limited and they are often combined with a carbonic anhydrase inhibitor. Timolol is the agent of first choice although bradycardia and bronchospasms are some of the side effects reported. Betaxolol has less side effects because of its β_1 blocker specificity (Renwick, 2014).

5.2. Carbonic Anhydrase Inhibitors (CAI)

Carbonic anhydrase is the enzyme involved in the formation of carbonic acid from carbon dioxide and water and for this direct reason their effect can be the reduce of IOP levels by up to 50% (Martín, 2017). They are used in long term medical management and are available both in topical (dorzolamide, brinzolamide) and systemic formulations (acetazolamide, methazolamide). The systemic CAI have important side effects such as potassium depletion, metabolic acidosis, diuresis, anorexia and gastrointestinal disturbances (vomiting and diarrhoea) and for all of these the topical CAI are preferred (Renwick, 2014). Acetazolamide may be used intravenously in emergency cases. Dorzolamide and brinzolamide are the topical CAI most frequently prescribed and they both have a similar maximum effect peak at around 6 hours after treatment, suggesting that an 8 hours interval of administration is the optimal regime (Alario, 2015).

5.3. Prostaglandin Analogues (PGA)

These synthetic derivatives of prostaglandin F2α increase the uveoscleral outflow by mechanisms not completely understood but which are thought to be related to the hydrolysis of the extracellular matrix within the muscle fibres of the CB by the activation of metalloproteinases (Martín, 2017). Latanoprost and travoprost are the most commonly used. Depending on the study, once a day application of latanoprost reduced IOP by 22% to 40% of normotensive dogs of various breeds and by 50% in laboratory Beagles with POAG (Alario, 2015). PGA appear to be ineffective in cats

because of the lack of specific receptors although they are able to cause miosis in both dogs and cats (Renwick, 2014). Travoprost and bimatoprost also lack a generic formulation, opposed to latanoprost, therefore having a prohibitive cost, tending to be replaced by the latter (Alario, 2015). Because of its miotic effects they are contraindicated in most cases of glaucoma induced by any blockage of the pupil, which is exacerbated by miosis (Clode, 2018)

5.4. Osmotic agents (Mannitol, Hydroxyethyl Starch, Glycerin)

The use of osmotic agents is often reserved for acute and emergent glaucoma cases with IOP superior to 40 or 50 mmHg because of their rapid action in creating an osmotic gradient capable of reducing the ultrafiltration process (Martín, 2017; Renwick, 2014). Mannitol is the most powerful drug and is usually given in a 20 minutes IV perfusion in the dosage of 1-2 g/kg (Martín, 2017). Since mannitol causes dehydration, caution with its use is needed in animals with cardiovascular and renal diseases (Clode, 2015).

5.5. Special medical treatment considerations in cats

Although similar, the treatment of glaucoma in cats has a poorer outcome because of late presentation. The owner compliance is also questionable for the fact that topical medications often have a nasolacrimal drainage and have an aversive taste (McLellan, 2014). The occlusion of the lacrimal puncta with digital pressure in the moment of the medication application may lessen this fact (Renwick, 2014).

Regardless of the underlying cause, topical CAIs are usually the first choice since dorzolamide proved to diminish IOP in both normal and glaucomatous cat eyes opposed to brinzolamide which seems to have less effect (McLellan, 2015).

Most of the PGA available do not have a significant effect in normal cats except for their strong miotic effect which limits its use in cases of severe uveitis (Renwick, 2014).

The miotic effect of β -blockers should also be considered in cases of uveitis and timolol are contraindicated in cats with feline asthma or cardiac disease due to the bradycardia and bronchoconstriction they promote (McLellan, 2015).

Glaucoma should always be confirmed in case of uveitis presentation because topical steroids were proven to elevate IOP in normal cats (Renwick, 2014).

6. Neuroprotective agents

The advances in neuroprotective investigation claims the use of neurotrophic factors as well as Brain-Derived Neurotrophic Factor (BDNF) and neuroprotective agents such

as glutamate inhibitors, NMDA-receptor antagonists, NMDA-like antagonists and calcium channel blockers.

BDNF is physiologically secreted by nervous tissue and transported via the axoplasms into the retina where it prevents the apoptosis of the RGC. The intravitreous injection of this factor is being under study (Martín, 2017).

Riluzole acts by blocking glutamate channels and therefore diminishing their cytotoxic activity (Renwick, 2014).

Nemantine, a NMDA receptor antagonist and flupirtine, an NMDA-like antagonist, aim to reduce the free radical effects, protecting cells from tissue necrosis and cellular apoptosis (Martín, 2017).

The use of amlodipine and flunarizine, both calcium channel blockers and antihypertensives are used due to their property of reducing the systemic arterial blood pressure and increase blood flow velocity through the short posterior ciliary artery, long posterior ciliary artery and ophthalmic artery (Gellat, 2013).

According to Resende, 2018, erythropoietin has also proved to have a protective action on the retina due to the anti-inflammatory, anti-apoptotic and anti-oxidative properties on the RGC and RPE and for the recruitment of endothelial cells to protect the retinal vasculature.

Other drugs and nutraceuticals such as vitamin E, melatonin and tirilazad mesylate act by blocking lipidic peroxidation by free radicals (Martín, 2017).

7. Genetic therapy

The attempts of over expressing genes which code for the family of antiapoptotic proteins bcl-2 is still far from being a reality due to the difficulty in maintaining in a sustained manner their presence in the RGC (Martín, 2017).

8. Surgical Treatment

The purpose of all the surgical techniques is to maintain the glaucomatous eye visual for as long as possible and to relieve the pain. Therefore, general considerations for animals receiving antiglaucoma drugs should be addressed.

Before the induction of anaesthesia, blood gas and acid-base analysis should be performed, as well as the measurement of serum potassium levels, especially in those being medicated with CAI and the rehydration should also be guaranteed in those who had received mannitol (Miller,2013).

Regarding visual glaucomatous eyes, the aim in surgical treatment is to decrease IOP through filtering or through cyclodestructive procedures. For blind globes, to control or prevent ocular discomfort by means of enucleation or evisceration is the end-stage recommended procedure.

Table 6 – Recommended surgical procedures for visual and for blind eyes

Visual eye

• Increase of the aqueous humor outflow
  • Gonioimplantation
  • Other filtering procedures
• Decrease aqueous humour production
  • Cyclodestructive techniques
  • Transcleral Cyclophotocoagulation
  • Endoscopic Cyclophotocoagulation

Blind eye
• Chemical Ablation of Ciliary Bodies • Enucleation • Evisceration (with or without intrascleral prosthesis)

8.1. Visual Eyes

8.1.1. Gonioimplants

The best candidates for gonioimplants are visual eyes with a solo acute glaucoma episode, the ICA is closed, narrowed or goniodysplasic, have IPO inferior to 30 mmHg and there is no Tyndall effect (Martín, 2017). Its use in blind eyes or with intraocular neoplasia are contraindicated (Miller, 2013).

Gonioimplants are devices which promote the elimination of the AH by diverting its flow through a drainage tube into various anatomic sites like the frontal sinus, the parotid salivary duct, the nasolacrimal duct or the orbit (Miller, 2013).

They can be divided in two types, those with a pressure-sensitive valve (Ahmed®, Krupin-Denver®, Joseph®, Cullen®) and those without it (Molteno®, Baerveldt®, Ex-Press®, SOLX Gold®). The valves are unidirectional, allowing the AH to flow from the anterior chamber into the filtration bleb in only one direction. The implants which lack a valve allow the AH flow in both opposite directions and although being cheaper the risk of developing endophthalmitis is higher (Martín, 2017).

The most frequent complications of this technique include: intraoperative intraocular haemorrhage, postoperative uveitis, obstruction of the tubing with fibrin immediately in the postoperative period, implant loosening, the formation of scar tissue in the filtering bleb usually 6 to 12-month postoperative (Renwick, 2014). The deposition of fibrin may be decelerated with intracameral injection of tissue plasminogen activator (TPA) and the use of anti-fibroblastic agents such as mitomycin C or 5-fluorouracil may limit fibrosis and scarring (Miller, 2013). It is also frequent the need to add medical treatment after a few months of surgery such as latanoprost or dorzolamide (Martín, 2017).

8.1.2. Other filtering procedures

Techniques such as scleral trephination and peripheral iridectomy were historically used to increase the outflow but the formation of drainage holes through the sclera has proven to have a high risk of uveitis and scarring, leading frequently to failure (Renwick, 2014).

8.1.3. Cyclodestructive techniques

The purpose of these techniques is to promote the ablation of the CB processes in order to diminish the AH production.

8.1.3.1. Laser Cyclophotocoagulation (CPC)

One of the forms of destroying CB processes is to irradiate it with a diode laser (more common) or a Nd:YAG laser, through the sclera (trans-scleral CPC) or within the eye (endoscopic CPC) (Miller, 2013). The absorption of the energy at the melanin deposit sites in the CB epithelium causes coagulation and necrosis of the CB processes and surrounding tissues (Martín, 2017). Frequently more than one treatment is needed (Miller, 2013).

The trans-scleral protocol uses 30-50 treatments at multiple sites, 3-4 mm behind the limbus and avoiding the 3 and 9 o'clock positions in order to prevent any damage to the long posterior ciliary arteries (Renwick, 2014).

Endoscopic CPC is completely similar to the previous but inserts a fine diode laser probe with a camera, within the globe via two limbal incisions, through the pupil and under the iris (Renwick, 2014). The damage to the CB processes achieves a better control but the risk of significant uveitis and the invasiveness of the procedure requires a highly skilled ophthalmic surgeon (Martín, 2017).

8.1.3.2. Cyclocryotherapy

Cryogens such as liquid nitrogen or nitrous oxide are used to induce necrosis of the CB using cryoprobes on six to eight spots of the CB processes, frozing them for about two minutes (Miller, 2013). Uveitis, chemosis, neurotrophic keratitis when the long posterior ciliary nerves are damaged may be expected as well as IOP spikes that can persist for days after surgery (Bras, 2015). Additional aqueocentesis may be needed to control IOP levels and increases in the risk of uveitis, endophthalmitis or damage to the lens (Miller, 2013).

8.2. Blind Eyes

8.2.1. Chemical Ablation of the CB

Intravitreal gentamicin or cidofovir injection may be used to destroy pharmacologically the CB epithelium (Maggio, 2015). Cidofovir is usually preferred over gentamicin in patients with renal disease (Oliver & Smith, 2014). The main recommendations for these procedures are patients with high anaesthetic risks or those who have financial constraints (Miller, 2013).

Chemical ablation can induce intraocular inflammation, haemorrhage, corneal opacity, cataracts, *phthisis bulbi* and intraocular tumours namely sarcoma formations in cats (Maggio, 2015; Oliver & Smith, 2014).

8.2.2. Enucleation and Evisceration

The major indications for enucleation include blind eyes with medically uncontrolled IOP, failure in pain management, exposure complications due to buphthalmia and primary neoplasia (Renwick, 2014). After the removal of the ocular globe, the use of polymethylmethacrylate or silicone prosthetics may be inserted for a better cosmetic result (Maggio, 2015).

In a nonneoplastic eye in which the ocular surface is healthy and intact, an evisceration with the dissection and removal of choroidal and uveal tissues can be performed, with the insertion of an intrascleral prosthesis (Maggio, 2015).

In cases of visual eyes with invasive or infiltrative masses, enucleation or orbital exenteration are the indicated procedures (Donaldson, 2014).

9. Drug effects on IOP

Animals with a suspected or confirmed glaucoma diagnosis who are presented for ophthalmic surgery may represent a challenge to the anaesthetist due to the anaesthetic drugs effects on IOP. Other severe ocular conditions in which a complete rupture of the globe integrity is at stake, such as descemetoceles, deep corneal ulcers or trauma should also benefit from a more careful selection of anaesthetic agents. For these reasons, anaesthetic management should be aimed at minimizing increases in IOP over the anaesthetic period in both ophthalmic and nonophthalmic surgeries.

The mechanisms which lead to increases in IOP are mostly derived from some of the anaesthetic agent's side effects. For the purpose of this dissertation, only the premedication agents are going to be considered.

Apart from the AH dynamics, the IOP is also determined by the choroidal blood volume, central venous pressure and extraocular muscle tone (Gross & Pablo, 2015). Most anaesthetics decrease IOP through a variety of factors including the depression of diencephalic centres which regulate IOP, the relaxation of extraocular musculature or directly by increasing AH outflow or decreasing venous and arterial blood pressures (Grimm, Tranquilli & Lamont, 2011).

An increase in central venous pressure prevents the flow of AH into the venous system and this can be caused by excessive restraint of the head and neck, coughing, retching and vomiting (Gross & Pablo, 2015).

When systolic arterial blood pressure decreases below 90 mmHg, the blood volume of the intraocular choroidal circulation also decreases leading to a marked IOP reduction (Cunningham & Barry, 1986).

The increase in the arterial partial pressure of carbon dioxide ($PaCO_2$) and decrease in the arterial partial pressure of oxygen (PaO_2) result both in hypercapnia and hypoxemia, respectively, inducing vasodilation which rises choroidal blood volume and consequently IOP (Gross & Pablo, 2015).

Preanesthetic agents can be useful to reduce stress and anxiety, to facilitate restraint, decrease the dose requirement of potentially more dangerous drugs used in general anaesthesia, to smooth the induction phase, to enhance perioperative analgesia, and to reduce arrhythmogenic autonomic reflex activity (Grimm, Tranquilli & Lamont, 2011).

The most common preanesthetic agents' categories are briefly described in the following pages.

9.1. Tranquilizers and sedatives

9.1.1. Phenotiazines: antagonists of D2 dopaminergic receptors which decrease neurotransmission as well as having an antiemetic effect at the chemoreceptor trigger zone. They are also responsible for blocking norepinephrine at α-adrenergic receptors (α-1 antagonism) resulting in peripheral vasodilation, systemic hypotension and a decrease in cardiac output (Posner, 2018). Examples of these drugs are acepromazine, promazine, chlorpromazine.

9.1.2. Benzodiazepines: are commonly used as antianxiety agents, anticonvulsants, muscle relaxants and sedatives/hypnotics (Brutlag, 2014). They bind to the gamma subunit of the gamma-aminobutyric acid receptor subtype A ($GABA_A$). that can be primarily found in the cerebral cortex and with very few receptors outside the CNS, hence their minimal cardiopulmonary effects (Posner, 2018). Examples of these drugs include diazepam, midazolam, lorazepam and zolazepam.

9.2. Anticholinergics

The blockage of the M_2 muscarinic receptors in the areas innervated by the vagus nerve can reverse the bradycardia induced by the vagal tone from the sinoatrial and atrioventricular nodes (Duke-Novakovski, 2016). Anticholinergics can be administered to reduce salivation induced by ketamine (Kastner, 2016). Examples of these agents are atropine and glycopyrronium. Atropine is a potent mydriatic and cycloplegic drug that can interfere with ocular lens accommodation and is contraindicated in all cases of glaucoma, except those caused by iris bombé (Renwick, 2014; Duke-Novakovski, 2016).

9.3. Analgesics

9.3.1. NSAIDs

NSAIDs are anti-inflammatories which provide good analgesia by inhibiting cyclo-oxygenase enzymes and thus the formation of inflammatory prostaglandins. Topical NSAIDs such as ketorolac and flurbiprofen are frequently used to control miosis and inflammation perioperatively in intraocular surgery (Jolliffe, 2016).

9.3.2. Opioids

The most important advantages of the use of opioids are the potent analgesia and sedation they provide without direct myocardial depression, although they do depress the medullary ventilatory control centres which leads to a decreased responsiveness to high carbon dioxide levels and, in return, to hypoventilation (Pascoe & Steffey, 2018).

This class of drugs have very few negative effects on haemodynamics. The stimulation of the vagus nerve may cause a decrease in heart rate which can be managed by co-administration of an anticholinergic agent, if necessary (Murrell, 2016). Most opioids have minimal effects on cardiac output in dogs, except for methadone which may produce more consistent decreases (Pascoe & Steffey, 2018).

Opioid receptors have three major classes of receptors μ, κ, and δ with particular effects (table 7) and they also may be full agonists, partial agonists, antagonists and combinations thereof.

Table 7 - Opiate receptor types and their associated effects, Adapted from KuKanich & Papich, 2018

	μ Receptor	**κ Receptor**	**δ Receptor**
Analgesia	√	√	√
Respiratory depression	√		
Decrease gastrointestinal motility	√	√	
Sedation	√	√	
Euphoria	√		
Miosis/mydriasis	(species specific)	(species specific)	
Nausea/vomiting or antiemetic	(drug specific)	(drug specific)	

The choice and dosage of the appropriate opioid depends upon the anticipated level of pain caused by the lesion and/or procedure and its duration (Jolliffe, 2016). The agonists produce a dose-dependent effect which reaches a plateau with unconsciousness (KuKanich & Papich, 2018).

Methadone has a higher affinity for the **μ**-opioid receptor than morphine and is also antagonist at the N-methyl-D-aspartate receptor as well as a norepinephrine and serotonin uptake inhibitor (Duke-Novakovski, 2014). The appropriate routes of its administration in cats and dogs are intravenous, intramuscular and subcutaneous and,

unlike morphine, methadone rarely induces emesis which can be useful in patients with increased intraocular or intracranial pressure (Kerr, 2016).

9.4. Dissociative agents / N-methyl-d-aspartate (NMDA) receptors antagonists

These drugs prevent the binding of excitatory neurotransmitters, glutamate, and glycine at the NMDA receptors, preventing the conduction of ions (Na^+, K^+, and Ca^{2+}) (Posner, 2018). This characteristic inhibits the firing of the second-order neurons which depress the activity at the thalamocortical and limbic systems as well as the nuclei in the reticular activating system (Love & Thompson, 2018). Ketamine, one of the commonest dissociative agents, also promotes profound analgesia and amnesia with maintained ocular, laryngeal, pharyngeal, pinnal and pedal reflexes and increased muscle tone, muscle spasm and seizures (Kastner, 2016). The use of ketamine alone significantly increases IOP, being the contraction of the extraocular muscles the suggested mechanism (Thomson, 2007). Ketamine has also a stimulatory effect on the cardiovascular system, resulting in increased heart rate, arterial blood pressure and cardiac output (Kastner, 2016). The addition of a benzodiazepine or an alpha-2 adrenoceptor agonist is required to reduce these side effects (Posner, 2018).

9.5. Alpha-2 adrenergic agonists

The production of a reliable sedation and analgesia had made this group of drugs very popular in veterinary medicine. Adding to the fact of having their effects reduced by an antagonist like atipamezole, they also have a synergistic effect along with opioids or benzodiazepines which also make them a good rescue analgesic (Posner, 2018). The nonadrenergic imidazoline receptors can also be activated by some drugs in this group (e.g., medetomidine, dexmedetomidine and clonidine) which may be responsible for some of the hypotensive and antiarrhythmic effects (Seddighi, 2014). Respiratory and cardiac depression are usually a strong side effect of xylazine and dexmedetomidine, the last one being also responsible for a decreased cardiac output in consequence of intense vasoconstriction (Day, 2016).

Regardless of their cardiovascular properties, alpha-2 adrenergic agonists seem to decrease IOP in human eyes by decreasing the AH production and increasing uveoscleral outflow facility.

Alpha-2 agonists seem to also play a role in the degradation of the extracellular matrix material through the modulation of the expression and enzymatic activity of matrix

metalloproteinases and their tissue inhibitors in the outflow pathways, decreasing the resistance to uveoscleral outflow (Alario, 2015).

The blockage of the tonic effect of the sympathetic nervous system in the CBep is achieved by the activation of presynaptic alpha-2 receptors, which inhibits the release of norepinephrine (Alario, 2015).

The summary of the general haemodynamic effects induced by the group of drugs above described can be seen in table 8.

Table 8 – Summary of the general haemodynamic changes induced by drugs used in sedation in dogs and cats. NC=No change. Adapted from Duke-Novakovski, 2016

Drug	Heart Rate	Cardiac Output	Contractility	Vascular resistance	Blood pressure
Anticholinergic	↑	↑	NC	NC	NC or ↑
Phenothiazine	NC or ↑	NC or ↓	NC or ↓	↓	↓
Benzodiazepine	NC	NC	NC	NC	NC
Alpha-2 agonists	↓	↓	↓	↑	↑ then ↓
Opioids	↓	↓	NC or ↓	NC or ↓	NC or ↓
Ketamine	↑	↑	↑	↑	↑

Chapter III – Effects of methadone on intraocular pressure in dogs and cats – a pilot study

1. Objectives

The purpose of this study was to determine the effects of methadone on IOP after intravenous administration, as a solo drug of anaesthetic premedication, in dogs and cats.
The main answer sought is to discover if methadone may be a good choice as an anaesthetic premedication when it comes to intraocular surgery or in the need of sedation of glaucoma patients, if no interference in IOP can be proven.

2. Materials and methods

The present study was conducted between March and August of 2018, at the Teaching Hospital of the Faculty of Veterinary Medicine, University of Lisbon.

2.1. Animals Studied

The study group was composed of 32 dogs and 5 cats undergoing both ophthalmic or elective surgeries or even diagnostic procedures such as electroretinographies.
All the animals underwent a complete physical and ophthalmic examination before the drug administration.
The owners gave their consent for inclusion of their animals in the study.

2.2. Inclusion criteria

All the patients included in the study had a normal physical examination, normal CBC profile and biochemistry analysis (kidney and liver parameters) and also a completely normal ophthalmic examination, including normal IOP levels and no clinical signs of glaucoma at the time of admission to the consultation and at the beginning of the study.

2.3. Experimental protocol

IOP values were measured using a rebound tonometer (Icare ®, Helsinki, Finland) with each animal being positioned in a sitting position or in sternal recumbency, without e-collars and with the head maintained relaxed at the level of the thorax. The basal IOP of the patients was registered at the admission and before any type of restraint.

Animals were catheterized usually in the cephalic veins on the forelimbs or the lateral saphenous veins on the hindlimbs, using fixation wings intravenous catheters (Braun Introcan Safety ® W) and intravenous administration of methadone (Semfortan 10 mg/ml ® Esteve, Barcelona, Spain) at the 0,2 mg/kg dosage was then performed.

IOP variations were finally registered at ten (T10) and twenty (T20) minutes after the drug administration for all patients (figure 3).

Figure 3 – Measurement of IOP levels with the Icare ® tonometer at Faculty of Veterinary Medicine, University of Lisbon

The IOP values obtained were considered and registered when there was no superior difference of 3 mmHg within each measurement. The mean of the two values obtained was the final result considered in the statistical analysis.

3. Statistical data analysis

The data collected was registered and processed in Microsoft Office Excel (Microsoft Office ® 2011 for Mac) where some additional parameters as means and standard deviations were also calculated. The statistical data analysis was made using the software R ® 3.3.3 version with the R Commander extension. The statistical test elected was the Analysis of Variance (ANOVA) with one factor. This test could

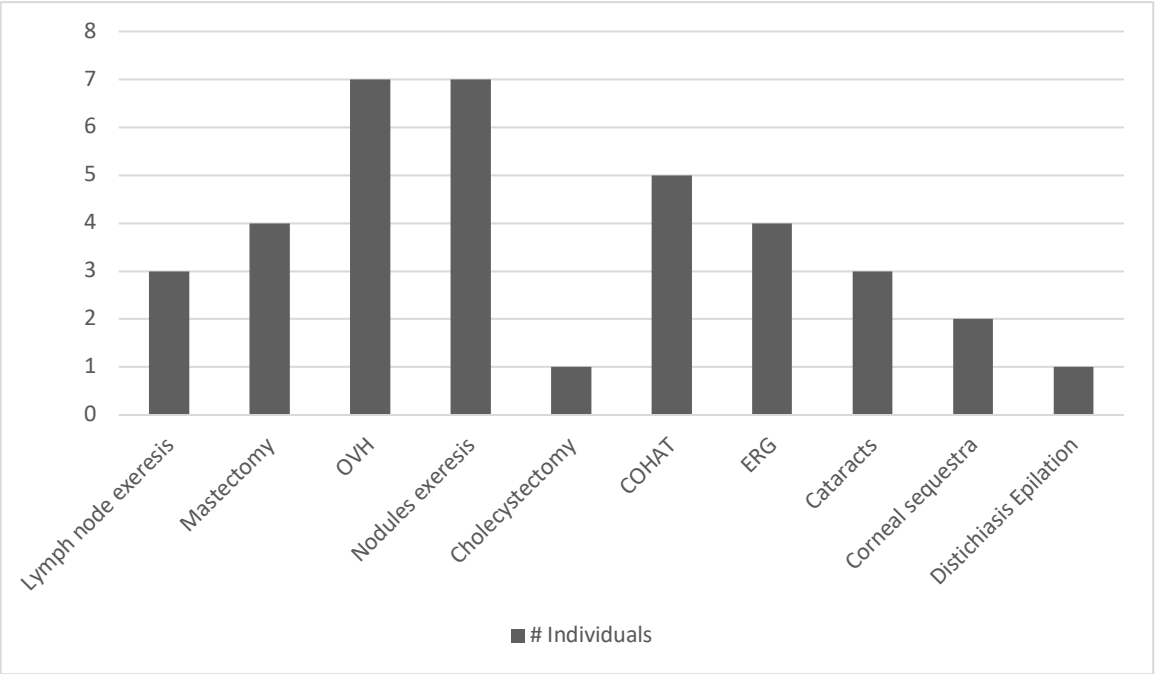
determine the possibility of existence of a significant difference between different times of treatment (T0, T10 and T20) within each individual eye.
All variables were then compared at each specific time point using a repeated-measures analysis of variance (ANOVA) and the differences were considered significant when P-value < 0.05 and non-significant when P-value > 0,05.

4. Results

4.1. Sample analysis

The group of 37 animals studied included 32 dogs of both sexes (14 males and 18 females) and 5 cats (3 males and 2 females) of 2 breeds. The mean ± SD ages of all the individuals were 8,2 ±3,73 having dogs' age ranged from 1 to 14 years and cats from 1 to 15 years.
The dogs' breeds corresponded to 20, including Maltese Dog (4/37), Yorkshire Terrier (3/37), German Shepherd (3/37), Mixed breed (3/37), Poodle (2/37), Portuguese Podengo (2/37), Pit Bull (2/37), French Bulldog (1/37), Rottweiler (1/37), Jack Russel Terrier (1/37), Cocker Spaniel (1/37), Boxer (1/37), Great Dane (1/37), German Spitz (1/37), West Highland White Terrier (1/37), Basset Hound (1/37), Staffordshire Terrier (1/37), Pointer (1/37), Fox Terrier (1/37) and Weimaraner (1/37).
The cats' breeds included European Shorthair (3/37) and Persian (2/37).
The procedures which required the use of methadone as part of the premedication or sedation protocol and in which the measurements were collected, are detailed in the graphic 3.

Graphic 3 - Absolute frequency distribution of the animals' procedures included in the study



4.2. Results of the ophthalmic examination

All the animals had a normal ophthalmic exam on what concerned ocular globe and nictitating membrane position, menace response, dazzle reflex, corneal and palpebral reflexes which were also tested. Schirmer tear test was not evaluated due to economic reasons and to minimize the restrain and further anxiety. The animals previously followed by an already ophthalmic existing condition had also been examined with a slit lamp biomicroscope and also with a panoptic ophthalmoscope.

4.3. IOP measurement results

The mean ± SD baseline (T0) and post-treatment (T10, T20) IOP values were respectively: 17,1 ± 3,32 mm Hg, 16,9 ± 3,37 mm Hg and 16,3 ± 3,33 mm Hg. In the majority of the animals, IOP levels decreased less significantly at T10 comparing to the mean values at T20. There were no statistically significant differences between baseline values and post-treatment values (p=0,296).

The results obtained from the 37 animals during the three moments of examination can be seen in graphic 4, where the IOP means as well as their respective standard deviations given by the lines extended vertically from the mean points are represented.

Graphic 4 - Variations of mean and standard deviation in thirty-two dogs and five cats sedated with methadone. Obtained from R ® R-Commander.

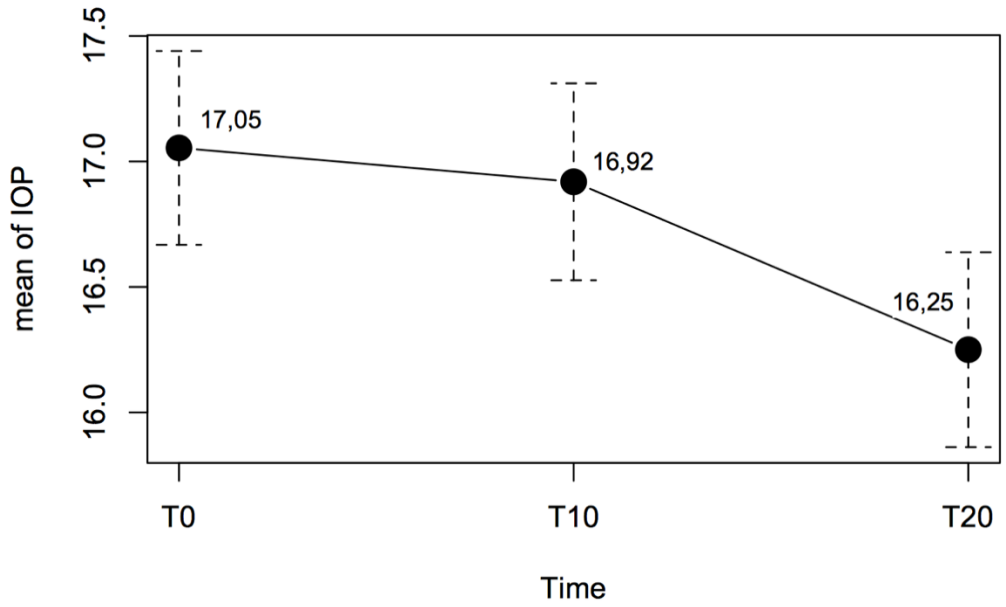


Table 9 - Variations of mean, difference between rows, total range and standard deviation in thirty-two dogs and five cats sedated with methadone

Time	Mean	Difference between rows	Total Range	Standard Deviation
T0	17.1	---	---	3.32
T10	16.9	-0,2	-0,2	3.37
T20	16.3	-0,6	-0,8	3.34

The IOP levels in the canine group did not suffer any increase, and the decrease of 0,6 mm Hg registered from T10 to T20 was more pronounced, than the 0,2 mmHg decrease in the period from T0 to T10. The plot of means can be seen in graphic 5 as well as the respective standard deviations, represented in table 10.

Graphic 5 - Variations of mean and standard deviation in thirty-two dogs sedated with methadone. Obtained from R ® R-Commander.

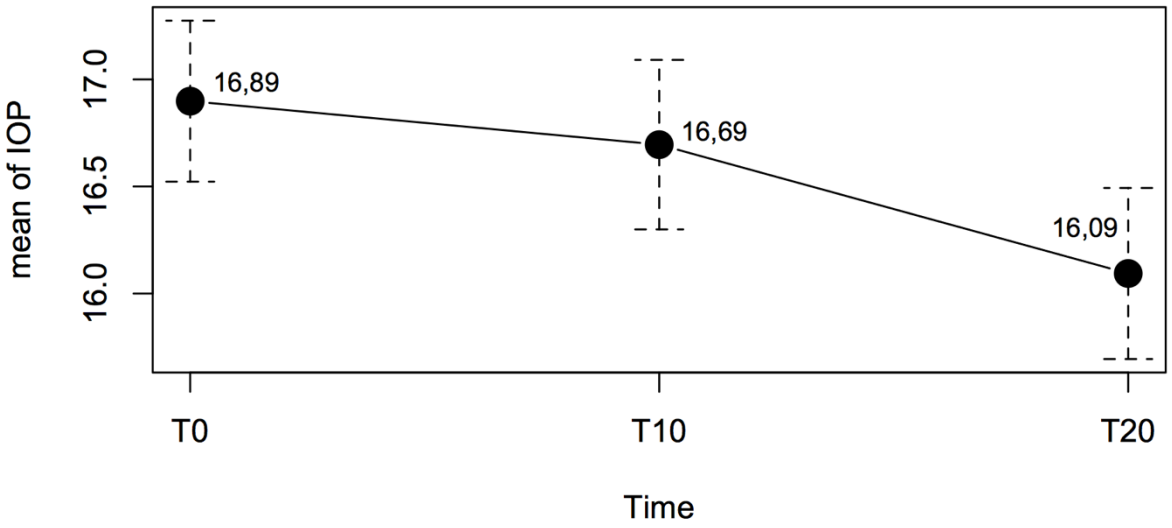


Table 10 - Variations of mean, difference between rows, total range and standard deviation in thirty-two dogs sedated with methadone.

Time	Mean	Difference between rows	Total Range	Standard Deviation
T0	16,9	---	---	3,00
T10	16.7	-0,2	-0,2	3,17
T20	16,1	-0,6	-0,8	3,19

Within the cat group, a transitory increase of 0,3 mm Hg in the mean IOP from T0 to T10 was observed. Apart from that fact, the total and final decrease of 0,8 mmHg from the beginning to the end of the experiment, i.e. from T0 to T20 was coincidently the same decrease found in the dogs' group.

The plot of means can be seen in graphic 6 as well as the respective standard deviations, represented in table 11.

Graphic 6 - Variations of mean and standard deviation in five cats sedated with methadone. Obtained from R ® R-Commander.

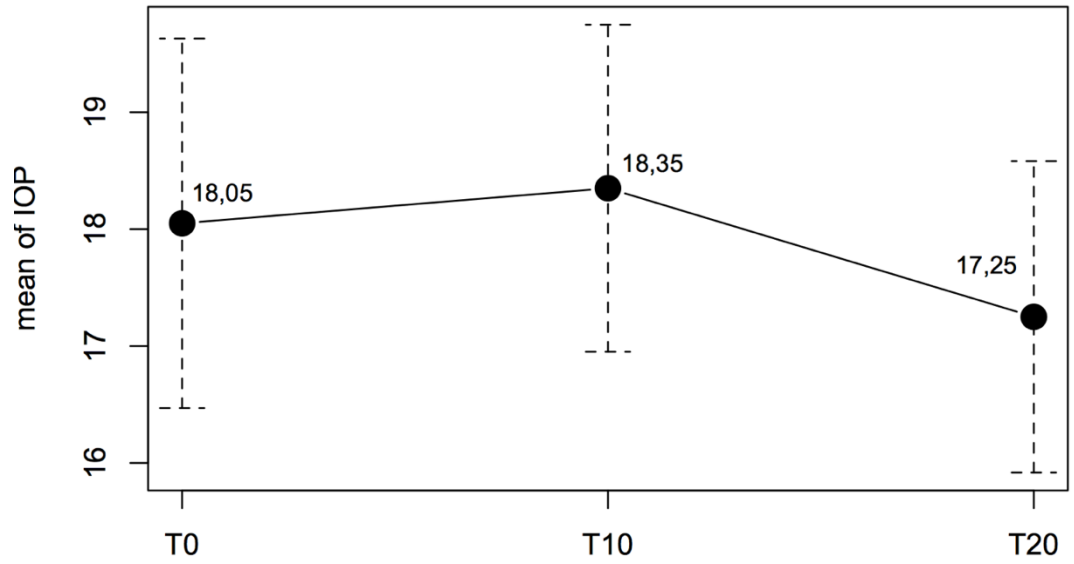


Table 11 – Variations of mean, difference between rows, total range and standard deviation in five cats sedated with methadone.

Time	Mean	Difference between rows	Total Range	Standard Deviation
T0	18,1	---	---	4,10
T10	18,4	+0,3	+0,3	4,42
T20	17,3	-1.1	-0,8	4,21

The three dogs submitted to cataracts surgery showed the mean and standard deviation values of 13,5 ± 0,9 mmHg, 12,8 ± 1,4 mmHg and 13,8 ± 1,7 mmHg at T0, T10, T20 respectively.

The two cats submitted to corneal sequestra surgical resolution presented with 23,3 ± 4,68 mmHg, 22,0 ± 3,06 mmHg and 21,3 ± 4,08 mmHg at T0, T10, T20 respectively.

A complete version of all the data collected from each individual can be seen in Annexe 1, page 55.

5. Discussion

5.1. Methadone as a premedication agent

The election of this particular opioid resided in the fact of being frequently used in most protocols of sedation and surgical pre-medication, for being an effective analgesic both in dogs and cats, even superior to butorphanol in the latter species (Steagall et al., 2006). Methadone, when used alone, produces minimal sedation (Monteiro et al., 2008) but for small diagnostic procedures such as electroretinographies the authors of the study found the level of sedation appropriate. Its combination with acepromazine can provide a powerful sedation, similar to morphine in dogs (Kerr, 2016) and during most of the surgical procedures of the animals included in the study also ended up adding acepromazine at the end of T20 because of the need of a more profound sedation. Apart from methadone, no other drugs were administered to the animals before all the measurements were collected.

The presence of opiate receptors has been suggested in the iris of rabbits and man; the intraocular injection of morphine was reported to induce a significant decrease in pupillary size of conscious rabbits as well as a pupillary constriction after a morphine conjunctival instillation in man (Drago et al, 1985). Drugs with sympathetic or parasympathetic actions may alter the IOP due to effects on aqueous humour formation, intraocular blood volume and vascular resistance and/or extraocular muscle tone (Gross & Pablo, 2015). According to Gellat, 2011, the different drugs used to tranquilize, sedate and/or anaesthetize may affect IOP directly by influencing the dynamics of the AH, or indirectly if hypercapnia or hypoxemia occurs or if changes in extraocular muscle tone are caused. The mechanisms by which opioids lower intraocular pressure or may increase aqueous outflow drainage are still unknown, but hypothesis such as changes in the permeability of iris vessels or the inhibition of AH production at the CB are the ones considered by Drago et al, 1985.

Methadone, which is approved for use in dogs in some countries, appears to be well tolerated both by dogs and cats following intravenous administration, with the adverse effects reported with morphine such as nausea, vomiting, defecation, and dysphoria not being reported with methadone (KuKanich & Papich, 2018). Apart from the prominent sedation, a dose-dependent respiratory depression can occur but it is typically clinically insignificant within the appropriate dosages of 0,1-0.5 mg/kg in dogs (Kerr, 2016). Methadone has low oral bioavailability in dogs, although the terminal half-

lives range from 1.75–4 hours and 2–12 hours following intravenous (IV) and subcutaneous (SC) administration, respectively (Kukanich & Borum 2008).

The recommended methadone dose for intravenous administration in cats is not consensual, ranging from 0,2-0,4 mg/kg (Maddison & Murrell, 2013) and 0,1-0,3 mg/kg (Kerr, 2016) but it appears to cause less excitation or vomiting than some other μ agonists (Maddison & Murrell, 2013). The frequency of administration of these doses based on pharmacokinetics were not evaluated in cats but based on clinical observations, the interval of 6–8 hours is suggested by some authors (KuKanich & Papich, 2018).

5.2. Methadone effects on IOP

In the current study, the sedative effect of methadone did not alter the ocular globe and nictitating membrane position as well as the menace response, dazzle reflex, corneal blink reflex or palpebral reflexes. None of the animals studied presented the described clinical signs related to opioids such as salivation, nausea, vomit, defecation, dysphoria or a clear modification of the respiratory pattern, probably because the administered dosage was the lowest recommended.

Some parameters could have also represented a valuable contribution to this study such as the pupil diameter size, using a pupillometer, in order to exclude the possibility of having some degree of pupillary constriction that could alter AH outflow facilities. The inexistence of such expensive device in our facilities made its inclusion impossible. IOP values could also be correlated with continuous measurements of respiratory rates, pulse and heart rate, as well as monitoring oxygen saturation of haemoglobin (SpO2) following sedation to try to evaluate respiratory depression ascribed to opioids. These parameters were also not evaluated due to difficulty in obtaining reliable results for having the necessity of continuously restrain the patients during at least the twenty minutes of the study, especially in the latter parameter.

The results achieved in this study concluded that the mean IOP levels both in dogs and cats did not suffer significant increases, with the only exception of the increase of 0,3 mm Hg in the mean IOP from T0 to T10 in the cats group. In addition to the cats' group, the total and final decrease of 0,8 mmHg from T0 to T20 was exactly the same final decrease in the dogs group and in the same time interval. The IOP levels in the dogs group did not suffer any increase, and the decrease registered was more notorious from T10 to T20, with a decrease of 0,6 mmHg, compared to the 0,2 mmHg decrease in the period corresponded to T0 to T10.

The fact that the standard deviations may be considered high in both groups, reflects only the fact that all the individuals have IOP levels that are highly variable within each other. Furthermore, it is expected that the data variability tends to decrease in large sample sizes, therefore explaining the reason for the wide distribution of values since the sample size of the study is rather small.
In this study neither statistically nor clinically significant differences occurred between left and right eyes.

The animals included in the study which were submitted to COHAT (Comprehensive Oral Health Assessment and Treatment) procedures were not diagnosed with any disease that could possibly affect any of the parameters of the ophthalmic exam analysed, such as retrobulbar masses or abscesses that could create fistulous tracts or other means of compression of the ocular globe.

The animals submitted to cataracts surgeries received eye drops topically immediately before the procedure as part of the protocol adopted by the lead surgeon. The application of these eye drops consisted in three cycles of one eye drop of Tropicamide (Tropicil Top ® Edol, Linda a Velha, Portugal), Ofloxacine (Oflex ® VAPP, Carnaxide, Portugal), Flurbiprofen (Edolfene ® Edol, Linda a Velha, Portugal) and Dexamethasone phosphate (Homatrocil ® VAPP, Carnaxide, Portugal), each applied at a four minutes interval.
It could be argued that the mydriatic activity of tropicamide and the topical use of NSAIDS as well as corticosteroids could significantly elevate the IOP levels, although no evidence of this occurrence was found in this study. According to Pumphrey, 2015, there is a possible corticosteroid-induced or NSAID-induced ocular hypertension that could be considered and which may have the ability of increase IOP in susceptible dogs and cats, although their use is almost always necessary in cases of uveitic glaucoma. Mydriatic agents such as atropine and tropicamide have also the ability to increase IOP, although tropicamide is a weaker and therefore a safer agent than atropine (Oliver & Smith, 2014). On the opposite, some authors defend that mydriatic agents can lower IOP levels in very unique situations because of their ability of breaking down newly formed synechiae and preventing the formation of posterior synechiae thus releasing a pupillary block and promoting the restoration of the blood-aqueous barrier (Pumphrey, 2015).

In the present study, the group of dogs which underwent cataracts surgery were very small and therefore not representative. However, the total range in the variation of the three IOP means did not exceed 1 mmHg.

The group of cats which underwent corneal sequestra surgical resolution had a total range in variation of the IOP means of 2 mmHg, having high standard deviations at the same time. The fact that only two animals were studied and that the cornea defect could hinder a reliable IOP determination could explain the deviation.

In conclusion, the present study has shown that the sedative and analgesic effects resulting from the intravenous administration of 0.2 mg/kg of methadone did not appear to influence significantly the results in IOP levels neither in cats nor dogs. The awareness of the parallel effects that could be ascribed to the use of opioids such as methadone is important when considering its possible use not only in the need of intraocular procedures but even in its use as a unique sedative agent for an ordinary ocular examination of an animal with the suspicion or a confirmed glaucoma diagnosis.

5.3. Limitations of the study

The sedative effects of opioids, in this case methadone, may differ significantly depending on the dog's breed, character and level of excitement or aggressiveness. The great variability of all the individuals in terms of age, breed and temperament could also negatively affect the results obtained.

The moderately small sample size of the study decreases the strength of the obtained results because although they apparently confirm the hypothesis studied, the chances of assuming true a false premise are increased in small samples.

The absence of a control placebo group by ethical implications led to a prospective cohort study following only the variations in IOP individuals during time.

Chapter IV – Bibliography

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ANNEXES

Annexe I – Characterization of the parameters analysed in the population in study

						IOP Measurements						
#	**Spec**	**Breed**	**Sex**	**Age (yrs)**	**Procedure**		**T0'**	**Mean**	**T10'**	**Mean**	**T20'**	**Mean**
1	Dog	Maltese Dog	F	9	Cataracts	OD	12	**13**	13	**12**	13	**13,5**
							14		11		14	
						OS	15	**15**	11	**12**	14	**14**
							15		13		14	
2	Dog	Rottweiler	M	11	Lymph Nodes Exeresis	OD	16	**15,5**	14	**15**	15	**15**
							15		16		15	
						OS	12	**13**	14	**13,5**	12	**13**
							14		13		14	
3	Dog	German Shepherd	M	10	Lymph Nodes Exeresis	OD	11	**11,5**	13	**12**	12	**12**
							12		11		12	
						OS	14	**15**	16	**16,5**	14	**14,5**
							16		17		15	
4	Dog	Portuguese Podengo	M	7	ERG	OD	22	**20,5**	21	**21**	20	**21**
							19		21		22	
						OS	20	**19**	22	**20,5**	20	**21,5**
							18		19		23	
5	Dog	Maltese Dog	F	9	Cataracts	OD	15	**14**	16	**16**	17	**15,5**
							13		16		14	
						OS	13	**13**	14	**13**	16	**15**
							13		12		14	
6	Dog	Mixed breed	F	13	Mastectomy	OD	14	**15**	15	**15**	13	**13,5**
							16		15		14	
						OS	17	**17**	16	**16,5**	15	**15**
							17		17		15	
7	Dog	Poodle	F	11	OVH	OD	18	**19,5**	17	**18**	19	**18,5**
							21		19		18	
						OS	17	**16,5**	17	**17**	16	**16**
							16		17		16	
8	Cat	Persian	M	2	Corneal Sequestra	OD	24	**23,5**	22	**23,5**	24	**24**
							23		25		24	
						OS	27	**28**	27	**27**	26	**25**
							29		27		24	
9	Dog	Yorkshire Terrier	M	8	COHAT	OD	21	**20**	17	**17,5**	16	**17**
							19		18		18	
						OS	17	**17,5**	18	**18**	16	**16,5**
							18		18		17	

10	Dog	Boxer	M	6	Nodules exeresis	OD	16	**17**	17	**17,5**	17	**17**
							18		18		17	
						OS	20	**19,5**	18	**18,5**	18	**18,5**
							19		19		19	
11	Dog	Jack Russel Terrier	M	5	Cataracts	OD	16	**14,5**	12	**12,5**	10	**10,5**
							13		13		11	
						OS	12	**12,5**	15	**14**	11	**12**
							13		13		13	
12	Dog	Yorkshire Terrier	M	8	ERG	OD	19	**19**	13	**13**	12	**12,5**
									13		13	
						OS	17	**17**	18	**17**	17	**17,5**
									16		18	
13	Dog	Cocker Spaniel	F	9	ERG	OD	20	**20,5**	18	**18**	20	**20**
							21		18		20	
						OS	21	**23**	20	**21,5**	24	**23,5**
							25		23		23	
14	Dog	Mixed breed	M	14	Lymph Nodes Exeresis	OD	17	**16**	17	**16,5**	16	**15,5**
							15		16		15	
						OS	14	**14,5**	11	**11**	12	**11**
							15		11		10	
15	Dog	German Shepherd	F	5	Mastectomy	OD	21	**21**	22	**21,5**	19	**19,5**
							21		21		20	
						OS	15	**15,5**	17	**18**	17	**16**
							16		19		15	
16	Dog	Grand Danois	M	5	Distichiasis Epilation	OD	19	**18,5**	18	**17,5**	18	**17,5**
							18		17		17	
						OS	21	**21**	20	**20,5**	18	**18,5**
							21		21		19	
17	Dog	German Spitz	F	8	ERG	OD	13	**14**	13	**13**	12	**12,5**
							15		13		13	
						OS	14	**15**	13	**13,5**	14	**13**
							16		14		12	
18	Dog	Poodle	F	14	COHAT	OD	23	**22,5**	20	**19**	19	**20**
							22		18		21	
						OS	14	**15**	15	**14,5**	14	**13,5**
							16		14		13	
19	Dog	Westhighland White Terrier	M	8	COHAT	OD	17	**17,5**	16	**17**	14	**14,5**
							18		18		15	
						OS	19	**18,5**	20	**19**	17	**15,5**
							18		18		14	
20	Cat	European Shorthair	F	1	OVH	OD	15	**15,5**	15	**16**	16	**16**
							16		17		16	
						OS	14	**15**	17	**16,5**	16	**16,5**
							16		16		17	

21	Cat	Persian	M	3	Corneal Sequestra	OD	16	**15**	18	**19**	14	**15**
							14		20		16	
						OS	21	**23**	22	**20,5**	18	**18,5**
							25		19		19	
22	Dog	Basset Hound	M	10	Nodules exeresis	OD	22	**22,5**	26	**25**	24	**23**
							23		24		22	
						OS	16	**17,5**	19	**18**	17	**17**
							19		17		17	
23	Dog	Pit Bull	M	10	Nodules exeresis	OD	19	**20**	21	**22**	20	**19**
							21		23		18	
						OS	17	**17,5**	18	**18,5**	19	**18,5**
							18		19		18	
24	Dog	Maltese Dog	F	10	Nodules exeresis	OD	18	**18**	17	**17,5**	18	**17,5**
							18		18		17	
						OS	15	**14**	14	**15**	15	**14,5**
							13		16		14	
25	Dog	Pointer	M	8	Nodules exeresis	OD	20	**20,5**	19	**17,5**	17	**17,5**
							21		16		18	
						OS	23	**21**	20	**19**	17	**17,5**
							19		18		18	
26	Dog	Pitbull	F	10	Mastectomy	OD	15	**15,5**	16	**16**	13	**13,5**
							16		16		14	
						OS	19	**19**	21	**20**	21	**21**
							19		19		21	
27	Dog	Fox terrier	F	13	Cholecystectomy	OD	15	**14**	14	**13**	14	**14**
							13		12		14	
						OS	18	**18**	15	**15,5**	17	**17**
							18		16		17	
28	Dog	Mixed breed	F	1	OVH	OD	12	**12**	14	**14,5**	13	**13,5**
							12		15		14	
						OS	16	**16**	15	**16**	14	**14**
							16		17		14	
29	Dog	French Bulldog	F	4	Nodules exeresis	OD	15	**15**	15	**15**	12	**13**
							15		15		14	
						OS	21	**20**	17	**16,5**	17	**17**
							19		16		17	
30	Dog	Weimaraner	F	9	OVH	OD	23	**23,5**	24	**25,5**	24	**23,5**
							24		27		23	
						OS	18	**18,5**	19	**20**	20	**21**
							19		21		22	
31	Dog	Portuguese Podengo	F	2	COHAT	OD	15	**1**	15	**15,5**	15	**15**
							14		16		15	
						OS	17	**17,5**	20	**20**	18	**18,5**
							18		20		19	
32	Dog	Yorkshire Terrier	F	11	Mastectomy	OD	16	**17**	18	**19**	18	**17,5**
							18		20		17	
						OS	16	**16**	17	**17,5**	17	**17**
							16		18		17	
33	Cat	European Shorthair	M	15	COHAT	OD	15	**16**	18	**17,5**	15	**15,5**
							17		17		16	

						OS	17	**17,5**	17	**17**	15	**16**
							18		17		17	
34	Dog	Staffordshire Bull Terrier	F	3	OVH	OD	17	**17,5**	15	**16**	15	**15,5**
							18		17		16	
						OS	16	**16**	16	**17**	15	**16**
							16		18		17	
35	Dog	German Shepherd	M	11	Nodules exeresis	OD	10	**10,5**	11	**11**	9	**9,5**
							11		11		10	
						OS	12	**12,5**	13	**12**	13	**12**
							13		11		11	
36	Cat	European Shorthair	F	9	OVH	OD	15	**14**	15	**15**	15	**14,5**
							13		15		14	
						OS	12	**13**	11	**11,5**	11	**11,5**
							14		12		12	
37	Dog	Maltese Dog	F	12	OVH	OD	14	**14**	13	**13,5**	10	**11,5**
							14		14		13	
						OS	17	**16,5**	15	**16**	15	**15,5**
							16		17		16	

OD = Oculus dexter (Right eye); OS= Oculus sinister (Left Eye)

Annexe 2 – Abstract presented as Poster Presentation at the ESVO Annual Scientific Meeting 2018

EFFECTS OF METHADONE ON INTRAOCULAR PRESSURE IN DOGS AND CATS

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Purpose:
Determine the effects of methadone as a solo-agent of anesthetic premedication, on intraocular pressure (IOP) in dogs and cats undergoing both elective surgeries or other diagnostic procedures.

Methods: The study group was composed of 25 dogs and 3 cats with 8,2 ± 3,6 years. The baseline IOP (T0) of the subjects were registered approximately ten minutes after manipulation and placement of the iv-catheter in which methadone was given at the 0,2 mg kg^{-1} dose. The variations were then registered after ten (T10) and twenty (T20) minutes. IOP values were measured with Icare® rebound tonometer and each animal was positioned in sternal recumbency, without e-collars and with the head maintained relaxed at the level of the thorax.

Results:
The mean ± SD baseline (T0) and post-treatment (T10, T20) IOP values were respectively: 18,00 ± 5,69 mmHg, 18,50 ± 6,19 mmHg and 17.70 ± 6,0 mmHg. All variables were compared at each specific time point using one-way repeated measures analysis of variance (ANOVA). There was evidence of a statistical difference between baseline values and post-treatment values with $F(2,119)=8,286$, $p<0,05$.

Conclusion: IOP increased transiently at T10 in the majority of the animals but the mean values at T20 achieved lower values compared to T0. The variations found were statistically significant within each measurement, but not clinically significant, given that none of the animals suffered an increase above the physiological limit of 25 mmHg at the end of the experiment.

Support: UID/CVT/00276/2013.

Annexe 3 – Abstract presented as Poster Presentation for the CIISA Congress 2018

Effects of methadone on intraocular pressure in dogs and cats

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Background
The purpose of this study was to determine the effects of methadone as a solo-agent of anaesthetic premedication, on intraocular pressure (IOP) in dogs and cats undergoing both elective surgeries or diagnostic procedures.

Methods
The study group was composed of 32 dogs and 5 cats with 8,22 ± 3,73 years. Ophthalmic exam was normal. The baseline IOP (T0) of the subjects were registered before IV methadone at the 0.2 mg kg^{-1} dosage. IOP variations were registered ten (T10) and twenty (T20) minutes after the drug administration. IOP values were measured with rebound tonometry (Icare®, Helsinki, Finland) each animal being positioned in sternal recumbency, without e-collars and with the head maintained relaxed at the level of the thorax. All variables were compared at each specific time point using a repeated-measures analysis of variance (ANOVA) with R® 3.3.3 software and the R-Commander extension. The differences were considered significant when $P < 0.05$.

Results
The mean ± SD baseline (T0) and post-treatment (T10, T20) IOP values were respectively: 17,05 ± 3,32 mm Hg, 16,92 ± 3,37 mm Hg and 16,25 ± 3,33 mm Hg. IOP increased transiently at T10 in the majority of the animals but the mean values at T20 were lower than in T0. There were no statistically significant differences between baseline values and post-treatment values ($p=0.296$).

Conclusions
There were no significant variations in IOP values in dogs and cats after the administration of methadone as a solo-agent of anaesthetic premedication. Methadone may be a good alternative as anaesthetic premedication in intraocular surgery since it apparently does not interfere with IOP.

Keywords

Glaucoma, opioids, methadone

Funding Acknowledgements

Funded by Project UID/CVT/276/2013 (CIISA - Centre for Interdisciplinary Research in Animal Health).