Universitat Autònoma de Barcelona

The Effect Of Race And Selective Laser Trabeculoplasty On The Aqueous Humor Dynamics In Patients With Glaucoma

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TESI DOCTORAL

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Memòria presentada per
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ABBREVIATIONS

A	Area
ACD	Anterior chamber depth
ADP	Adenosine diphosphate
AFR	Aqueous flow rate
AH	Aqueous humor
AHD	Aqueous humor dynamics
ALT	Argon laser trabeculoplasty
ANOVA	Analysis of variance
AP	Pressure gradient
AQP	Aquaporins
ATP	Adenosine triphosphate
AV	Aqueous veins
AXL	Axial length
B	Ocular rigidity
BCE	Before the Common Era
C	Trabecular outflow facility
CC	Collector channel
CCT	Central corneal thickness
CDR	Cup-to-disc ratio
CE	Common Era
CH	Corneal hysteresis
CI	Confidence interval
CNTGS	Collaborative normal tension glaucoma study
CRF	Corneal resistance factor
CrI	Credible interval
dB	Decibel
EFA	Effective filtration area
EMGT	Early manifest glaucoma trial
EPV	Episcleral vein pressure
F	Force
Fin	Aqueous inflow

ABBREVIATIONS

FM	Fluorotron Master
Fout	Aqueous outflow
Ft	Aqueous flow rate
Ftrab	Trabecular outflow
Fu	Uveoscleral outflow
GAT	Goldmann applanation tonometry
gr	Gram
GSTT	Guy's and St Thomas' NHS foundation Trust
HBonf	Bonferroni method of analysis
IL	Interleukins
IOL	Intraocular lens
IOP	Intraocular pressure
IQR	Interquartile range
Ko	Loss coefficient
LASIK	Laser-assisted in situ keratomileusis
Max	Maximum
MD	Mean deviation
min	Minute
Min	Minimum
mJ	MilliJoule
mL	Millilitre
mm	Millimetre
mmHg	Millimetre of mercury
mW	MilliWatt
n	Number of subjects
Nd-YAG	Neodymium: yttrium aluminium garnet
NHS	National Health System
NPE	Non-pigmented epithelium
ns	Nanosecond
NTG	Normotensive glaucoma
OAG	Open angle glaucoma

ABBREVIATIONS

OHT	Ocular hypertension
OHTS	Ocular Hypertension study
ONT	Ocular normotensive (healthy volunteers)
P	Pressure
PACG	Primary angle closure glaucoma
PE	Pigmented epithelium
POAG	Primary open angle glaucoma
PT	Pneumotonometry
Pv	Venous pressure
R	Resistance
S	Surface tension
SC	Schlemm's canal
SITA	Swedish interactive thresholding algorithm
SLT	Selective laser trabeculoplasty
SS	Scleral spur
TM	Trabecular meshwork
TRT	Thermal relaxation time
µL	Microlitre
µm	Micrometre

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PROLOGUE

Why is glaucoma much more common and aggressive in patients of African descent? Unfortunately, we are not capable of answering this question just yet. Glaucoma is the leading cause of irreversible blindness worldwide and individuals of African descent are not only disproportionally affected by primary open angle glaucoma (POAG), the most common form of glaucoma, but also their disease develops earlier, presents with greater severity and progresses more rapidly. Thus, the management of this patients, at higher risk of blindless, is a challenge. Poor understanding of its aetiology has hindered attempts to early identification and treatment of disease. All studies agree that POAG is a complex and multivariate disease. This thesis has two parts and each, tries to answer a different question. First, we wanted to know whether African descent individuals had a greater imbalance in the production and outflow of the aqueous humor than white-Caucasians, since a greater aqueous production or a lower drainage could lead to a higher intraocular pressure (IOP). As of now, elevated IOP remains the only treatable factor of this disease. IOP can be reduced using medical treatment, surgically or with laser therapy. Selective laser trabeculoplasty (SLT), with a good safety profile and efficacy lowering IOP, has gained popularity during the last decade since its approval by the FDA in 2001. But… *how does SLT work? Does it increase the trabecular outflow?* We attempted to answer this question in the second part of this research.

This research was my introduction to the science of Aqueous Dynamics. It was an enriching learning experience that allowed me to understand glaucoma in a more "basic" level. I was able to use techniques such as Tonography and Fluorophotomtery which are otherwise not available in a normal clinical setting. The results from comparing the IOP, outflow facility and aqueous production between black and white individuals and the changes caused by SLT are presented and discussed in detail. At the end, the two papers published in two different peer-reviewed journals, resulting from this work have been attached.

INTRODUCTION

1. Anatomy of the ocular globe

The eyeball is covered by three layers or tunics (cornea and sclera, retina and uvea) and contains three compartments (anterior chamber, posterior chamber and vitreous cavity).

The outermost layer, the fibrous tunic is composed anteriorly by the cornea and posteriorly by the sclera. The *sclera* is an opaque white layer that covers the outer part of the globe, with an anterior opening for the cornea and a posterior one for the optic nerve. Not only protects the intraocular structures, but also maintains along with the intraocular pressure (IOP) the appropriate shape of the eye for a correct visual function. The thinnest and most fragile part of sclera is located just behind the insertion of the rectus (0.3 mm) reaching approximately 1 mm thick at the back around the optic nerve. The *cornea*, however, is a transparent and avascular tissue that occupies the centre of the anterior pole of the eye. This structure, convex on the outside and concave on the inside, has manly an optical function since it is the structure with more refractive power of the eye. The corneal thickness is approximately 0.7 mm in the periphery and 0.5 mm in the central area. The limbus, the transition zone between the cornea and sclera, is grey and translucent. In the innermost part of the corneoscleral limbus are the trabecular meshwork and Schlemm's canal.

The vascular tunic or uvea is the middle layer of the eye and consists of the choroid, ciliary body and iris. It is characterised by being highly vascularised and pigmented. The *choroid* extends from the insertion point of the optic nerve to the limit with the ciliary body, the ora serrata. Its main function is to nourish outer layers of the retina. It consists of 3 layers of blood vessels: the innermost layer or choriocapillaris, a middle layer of small calibre vessels and the outermost layer of large calibre vessel. The blood supply to the choroid comes from the long and short posterior ciliary arteries and anterior ciliary arteries. The venous drainage is through the vortex veins. The *ciliary body*, of triangular section, serves as a bridge between the anterior and posterior segment of the eye. It has two main functions: the production of aqueous humor in the ciliary processes and visual accommodation. The ciliary body limits, anteriorly, with the iridocorneal angle, contains the ciliary muscle and ciliary processes and limits posteriorly, through the pars plana, with the retina. The suspensory ligaments or

zonules join the ciliary body with the lens. Thus the contraction and relaxation of the ciliary muscle changes the curvature of the lens in the mechanism of visual accommodation. The *iris* is the most anterior uveal tract extension. It is a pigmented muscular structure that separates the anterior chamber from the posterior chamber. It has a ring shape with a central opening, the pupil, through which the aqueous humor synthesised by the ciliary processes passes from the posterior to the anterior chamber. The iris acts as a diaphragm regulating the amount of light that goes through the pupil and reaches the retina.

The sensory tunic is the innermost layer of the eye and consists of the retinal pigment epithelium and neurosensory retina where the photoreceptors are. The *retinal pigment epithelium* is a single layer of hexagonal cells extending from the optic nerve to the ora serrata where comes into contact with the pigmented ciliary epithelium. It is responsible for maintaining the blood-retinal barrier, absorbing the light scatter to avoid reflections and glare, and participates in the metabolism of photopigment and phagocytosis of unused segments of rods and cones. The *retina* is a transparent layer, located in the innermost part of the eyeball, weakly coupled to the pigment epithelium. The retina is organised in layers where the photoreceptors (rods and cones), interneurons and ganglion cells, whose axons form the optic nerve, are located.

1.1. Anatomy of the ocular chambers

In the eyeball there are two distinct chambers: the anterior and the posterior. The *anterior chamber* is a space limited anteriorly by the cornea and posteriorly by the iris and pupil. Laterally, the anterior chamber borders the front part of the ciliary body that forms the iridocorneal angle. In the emmetropic adult eye it has a volume of approximately 200 μL and a central depth of 3 mm, becoming shallower to the periphery. It is deeper in myopic eyes, aphakic and pseudophakic eyes and shallower in eyes with hyperopia. The *posterior chamber* is a space delimited by the iris and pupil anteriorly and the vitreous humor in the back, the ciliary body on the side and the lens in the medial part. These chambers are filled with aqueous humor, which is synthesised in the ciliary processes in the posterior chamber and flows through the pupil to the anterior chamber.

1.2. The Iridocorneal Angle

The iridocorneal angle is the area where the base of the iris and the peripheral cornea join. It includes the following structures: Schwalbe's line, trabecular meshwork (TM) and Schlemm's canal (SC), scleral spur (SS), base of the iris and ciliary body. The angle is where the aqueous humor evacuates the eye (**Figure 1**).

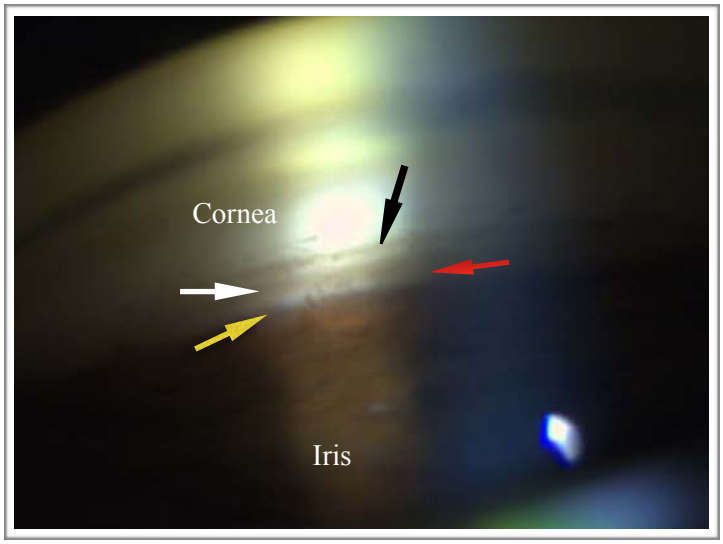


Figure 1. Anatomy of the iridocorneal angle. Gonioscopy of a pigmented iridocorneal angle where the Schwalbe's line (black arrow), trabecular meshwork (white arrow) scleral spur (red arrow) and ciliary body (yellow arrow) can be differentiated. Photo courtesy of Dr. Marcos Muñoz.

1.2.1. The Trabecular meshwork

The TM is a spongy-like structure located in the iridocorneal angle. It comprises sheets of thin connective tissue lined by endothelial-like cells, the *trabeculocytes*. Besides phagocytic properties, these cells also have contractile properties offering some resistance to the evacuation of aqueous humor.

The TM has a triangular cross section with the apex at the line of Schwalbe and the base formed by for the scleral spur and the ciliary body. It can be divided into three layers: the *uveal meshwork*, the *corneoscleral meshwork* and the *juxtacanalicular or cribiform meshwork* adjacent to the SC.

The *uveal meshwork* forms the lateral border of the anterior chamber, extending from the iris root and ciliary body to the peripheral cornea. It consists of bands of connective tissue covered by endothelial cells forming a three-dimensional mesh with large intercellular spaces that measure between 25 to 75 μm that offer a low resistance to aqueous humor outflow.

The *corneoescleral meshwork* is the intermediate zone and most extensive part of the TM. It is composed of a set of perforated lamellae of connective tissue covered by endothelial-like cells standing on a basal membrane. The lamellae are rich in glycoproteins, collagen, hyaluronic acid and elastic fibres. The higher organisation of the corneoscleral meshwork in relation to the uveal meshwork, as well as their narrower intercellular spaces are responsible for the increase in flow resistance (*Lütjen-Drecoll & Rohen*, 1994).

The *cribiform or juxtacanalicular meshwork*, the outermost part of the TM, is in direct contact with the inner wall endothelial cells of SC. Instead of connective tissue beams, the juxtacanalicular region is an open connective tissue matrix in which fibroblast-like cells are located. The extracellular matrix composed of a variety of macromolecules such as hyaluronic acid, glicosaminoaglicans, collagen, fibronectin, laminin and other glycoproteins produced by the surrounding cells. The cells form long processes by which they attach to each other, to the extracellular matrix and to the inner wall of the SC. (*Gong et al.*, 1996) This is the layer with smallest intercellular spaces offering the majority of the tissue resistance to outflow.

1.2.2. Schlemm's canal

The SC is a circular tube similar to a lymphatic vessel that runs through the iridocorneal angle. Its inner wall of endothelial cells is in direct contact with the cribiform meshwork, and both together provide the maximum resistance to the passage of aqueous humor.

The canal is the last barrier the aqueous humor must cross before exiting the eye. (*Lütjen-Drecoll & Rohen*, 1994)

1.2.3. Collector Channels and Aqueous Veins

Aqueous flow into SC is not evenly distributed throughout the inner wall but occurs preferentially in certain areas. Drainage of aqueous humor preferentially occurs near *collector channels* (CCs) in the human eye. CCs are randomly distributed around the eye with a higher distribution on the inferior-nasal side than on the temporal side of the

eye. From the CCs, aqueous humor passes through a tortuous system of passages termed the deep, midlimbal, and superficial intrascleral venous plexus that lead in turn to episcleral veins that return the blood to general circulation (*Gong & Francis*, 2014).

The *aqueous veins* (of Ascher) are in direct contact with the collector channels and episcleral veins bypassing the deep and intrascleral venous plexus. Aqueous veins (AVs) contain clear aqueous at their origins, but since they anastomose with episcleral vessels that contain blood, transitional zones in AVs can be identified on the conjunctival surface as a large vessel with a clear central lumen bordered on either side by dark blood. AVs vary en size, position, number and anatomical arrangement but are indistinguishable from conjunctival and episcleral veins (*Gong & Francis*, 2014).

2. The Aqueous Humor

Aqueous humor (AH) is the clear fluid that fills the anterior and posterior chambers of the eye and constitutes an important component of the eye's optical system.

2.1. Aqueous Humor Function

It is responsible for: 1) providing nutrients and oxygen to the avascular anterior segment consisting of the lens, corneal endothelium, trabecular meshwork 2) removing excretory products from metabolism; and 3) maintaining the inflation of the globe by keeping the intraocular pressure (IOP) relatively constant and within the physiological limits. Other functions are participation in local immune responses, delivery of antioxidants such as ascorbate and distribution of drugs to different ocular structures.

2.2. Aqueous Humor Composition

The composition of the AH depends not only on its production but also on the metabolic interchanges that occur within various tissues throughout its intraocular route. Arising from a plasmatic filtrate through fenestrated capillaries of the ciliary body stroma, the AH is actively secreted to the posterior chamber by the ciliary epithelium. AH is composed mainly of organic and inorganic ions, carbohydrates, gluthatione, urea, amino acids and proteins, oxygen, carbon dioxide and water. The composition of the AH

differs from the plasma composition due to the existence of the blood-aqueous barrier and the active transport through the ciliary epithelium. The greatest differences between the AH and plasma composition are the concentration of proteins and ascorbate.

The protein component of human AH is minimal, containing between 120 and 500 ng/μL of protein (*Goel et al.*, 2010) which represents 1% of the plasma protein concentration. The AH contains mainly low molecular-weight proteins such as albumin and B-globulins. The albumin is the most abundant protein (0,05 - 0,07 mg/mL) and represents half of the total proteins. (*Berman*, 1991) The most abundant immunoglobin is IgG (*Allansmith et al.,* 1973). The concentration of free aminoacids vary, but is higher compared to plasma, suggesting an active transport of amino acids through the ciliary epithelium (*Dickinson et al.*, 1968). The concentrations of Na^+, K^+ and Mg^{2+} in plasma and AH are similar. However, there is a deficit in Ca^{2+} and HCO_3^- and an excess of Cl^- compared to plasmatic levels. The AH is slightly hypertonic to plasma and this osmolarity is mantained in the anterior and posterior chambers. Glucose and urea in the AH are approximately 80% of the plasma levels. Glucose is of great importance since it provides energy to the retina and the avascular tissues such as the cornea and lens. The concentration of ascorbate though, is 20 to 50 times higher in the AH compared to plasma. Recently, evidence has been reported that ascorbate may be a regulator of ion channel activity, and not simply a scavenger of reactive oxygen species (*Nelson et al.*, 2007). In addition to the main components, the AH also contains different hormones, growth factors, neuropeptides, enzimes and citokines.

The maintenance of the composition of the AH is highly up-regulated since the clarity of the lens and cornea necessary for a good visual function depends on the correct interchange of water and solutes.

3. Aqueous Humor Dynamics

The study of the dynamics of AH goes back to classical times. Aristotle (384-322 BCE) was the first to record the watery contents that drained out of the eyeball. Celsus (53 BCE-7 CE) recognised two layers in the eye, the sclera/cornea and the choroid/ retina (arachnoide). The eye was filled with a glassy liquid called hyaloides. The

existence of an anterior chamber was mentioned first by Rufus of Ephesus (98-117 CE). Galen (131-201 CE) recognised there was an anterior and posterior chamber filled with fluid and pneuma separating the lens from the cornea. The AH nourished the lens, the organ of vision, therefore its loss was believed to lead to blindness. It was not until the 16th century that attention was paid to the regeneration of the AH. It was Volcher Coiter (1534-1576), in his *Tabulae oculorum humanorum* of 1573, the first to note that the aqueous replenished (*Mark*, 2010).

In the 19th century, Theodor Leber (1840–1917) proposed a model of the dynamics of AH flow that is in principle still held valid today. The AH would be produced mainly in the posterior chamber, move in bulk to the anterior chamber through the pupil and escape mainly through the TM to the SC and thence into the anterior ciliary veins. Three names are historically important: Hans Virchow (1852-1940), who described in detail the structure of the TM, Friedrich Schlemm (1795-1858), whose name is attached to the drainage channel, and Karl Ascher (1887-1971), who discovered the AVs. In 1941, while examining patients with a slit-lamp, Ascher detected superficial veins near the limbus carrying clear fluid. By pressing on the episcleral veins with a small glass rod confirmed the outflow of aqueous into the blood (*Mark*, 2010).

3.1. Aqueous Humor Production

The AH is secreted by the ciliary epithelium lining the ciliary processes. The ciliary body is divided in two parts, the *pars plana* and the *pars plicata*. The pars plicata, located more anteriorly, consists of some 70 villiform ciliary processes radiating inward toward the pupil. Each process is covered by the ciliary epithelium consisting of a non-pigmented epithelial (NPE) cell layer facing the posterior chamber and a pigmented epithelial (PE) cell layer located between the stroma and the non-pigmented layer. The stroma of each process contains loose connective tissue, a vascular core, and nerve endings from the posterior ciliary nerves. In the pars plana, the topography is flatter. At its most posterior limit, the ora serrata, the ciliary epithelium is fused with the sensory retina and the retinal pigment epithelium.

Before the AH is secreted to the posterior chamber the various components of the AH must traverse the capillary wall, the stroma and the epithelial bilayer of the

ciliary processes. There are three mechanisms involved in aqueous formation: **diffusion**, **ultrafiltration** and **active secretion**. *Diffusion* is the passive movement of solutes, especially lipid soluble substances, through the cell membranes proportional to a concentration gradient across the membrane (*Gabelt & Kaufman,* 2002). *Ultrafiltration* is the passive flow of water and water-soluble substances, limited by size and charge, across the fenestrated capillaries into the ciliary stroma, in response to an osmotic gradient or hydrostatic pressure. The hydrostatic pressure difference between the ciliary capillaries and IOP favours fluid movement into the eye while the oncotic gradient between the two resists fluid movement. Diffusion and ultrafiltration are responsible for the accumulation of the plasma ultrafiltrate in the stroma from where the AH is derived. This ultrafiltrate can only reach the posterior chamber by active secretion because of the tight junctions of the NPE cells that constitute the blood-aqueous barrier (*Uusitalo et al.*, 1973). *Active secretion* is an energetic consuming transport of water-soluble substances across the cell membrane against an oncotic pressure gradient; it is a complex process that accounts for the 80% to 90% of the total AH formation. The main site for active transport is believed to be in the NPE cells. However, gap junctions link adjacent cells within and between the two layers, so that the ciliary epithelium is a functional syncytium. There is an active transcellular transport of anions, cations, and other solutes across a concentration gradient in blood-aqueous barrier followed by an osmotic flow of water from the stromal interstitium to the AH. This active transport is mediated by protein transporters distributed in the cellular membranes and the energy required, is obtained from the hydrolysis of adenosine triphosphate (ATP) to adenosine diphosphate (ADP) by Na+-K+-ATPase, an enzyme located in both pigmented and non-pigmented ciliary epithelia. It involves mainly three steps: (1) uptake of water and solute at the stromal surface by PE cells through one of the two sets of electroneutral transporters, paired Na+/H+ and Cl-/HCO-3 antiports or Na+–K+–2Cl- symport, and cation-nonselective and tetrodotoxin-sensitive Na' channels, (2) flow of water and ions from PE to NPE through gap junctions, and (3) release of water and solutes from NPE cells into the AH in large part through membrane Na+-K+-ATPase (3 Na+ extruded in exchange for 2K+ taken up by the cell) and parallel K+ and Cl- channels, respectively. Cations, especially Na+, are also considered to cross between the cells through the tight junctions through the paracellular pathway in response to the small electrical driving force across the ciliary epithelium (about -1 mV). Aquoporins

(AQP1 and AQP4) are molecular water channels through which water is likely secreted (*Kleinzeller et al.*, 1997; *Goel et al.* 2010). Nutrients and other substances necessary for the survival of the lens and cornea are added to the fluid by the process of diffusion or facilitated transport. Although there is a net direction of secretion across the ciliary epithelium, there is also reabsorption of AH. Swelling-activation Cl^- channels, cyclic adenosine monophosphate, carbonic anhydrase and A3 Adenosine receptors are regulators of the aqueous formation (*Benos et al.*, 2008).

The mean aqueous flow in healthy eyes during wakefulness ranges from 2.2µl/min to 3.1µl/min (*McLaren*, 2009) and gradually decreases with age by about 2-3% per decade of life (*Brubaker*, 1991). This decrease in aqueous flow over the lifetime of an individual is statistically significant (*Becker*, 1958; *Brubaker*, 1981; *Brubaker et al.*, 1991; *Diestelhorst et al.*, 1992; T*oris. et al.*, 1999b) but is relatively small compared to age related changes in IOP and anterior chamber volume. (*Brubaker et al.*, 1981) From age 20 to 80 years, AH formation decreases by approximately 25% while anterior chamber volume decreases by 40%, resulting in 20% faster turnover rate of AH over a lifetime (*McLaren*, 2009). It follows a circadian rhythm, decreasing with sleep to 1.3 ± 0.4µl/min, approximately half the rate during daytime (*Reiss et al.*, 1984; *Brubaker*, 1991). This rhythm is not eliminated by sleeping under bright lights at night (*Koskela, et al.*, 1991), supine position during the day while awake (*Topper & Brubaker.*, 1985) or sleep deprivation (*Reiss et al.*, 1984). The mechanism that controls this biologic rhythm that has been of interest for decades remains poorly understood. Hormonal factors (*Kass & Sears*, 1977; *Brubaker*, 1991) that could drive the circadian rhythm and whether exists a negative correlation between IOP and aqueous flow have been studied extensively. Decreased aqueous humor formation with increased IOP, a phenomenon termed *"pseudofacility of Bárány"* (*Bill & Bárány,* 1966) has been shown to exist in anaesthetised monkeys and human volunteers but the effect is small and transient, indicating that rate of aqueous formation is relatively pressure-insensitive (*Moses et al.*, 1985; *Carlson et al.*, 1987). Pseudofacility was premised on the idea that raised IOP would diminish the pressure gradient for the ultrafiltration component of aqueous production. Patients with elevated IOP associated with glaucoma, ocular hypertension with and without pigment dispersion syndrome do not have reduced aqueous flow rates, and patients with reduced IOP associated with trabeculoplasty and miotonic

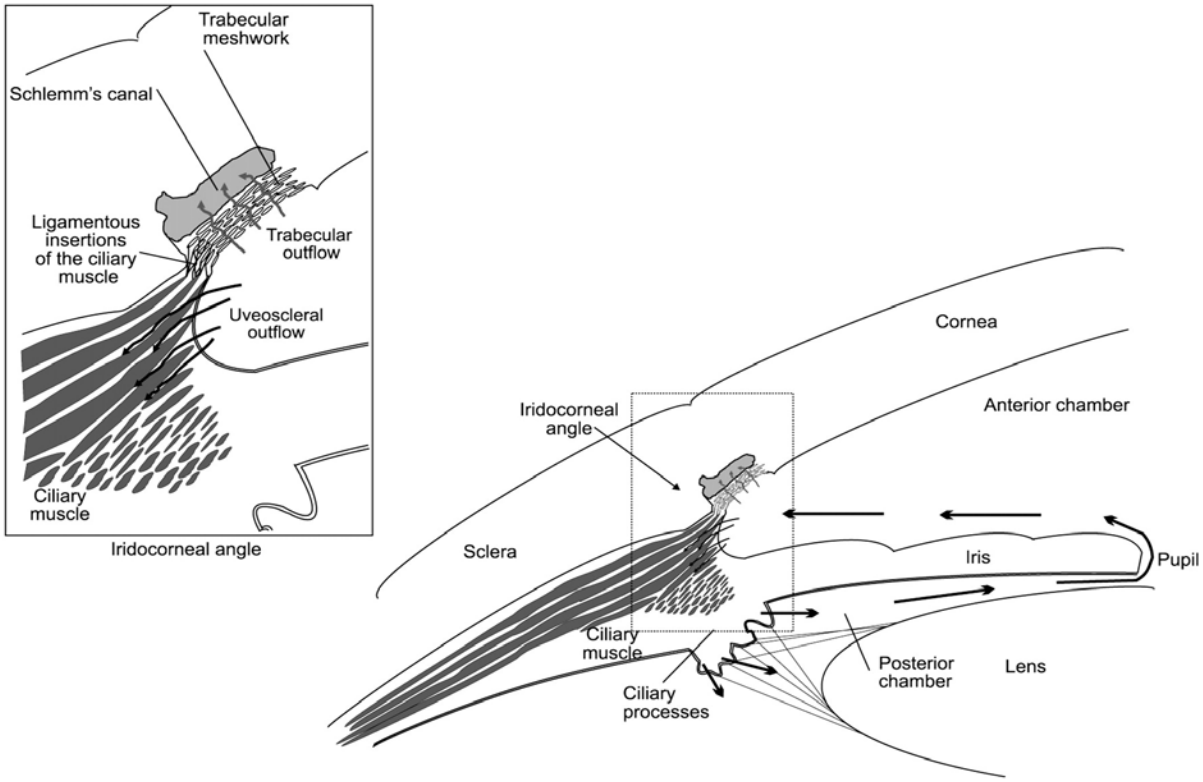


Figure 2. Aqueous humor outflow pathways: trabecular and uveoscleral. Aqueous humor exits the anterior chamber via two routes: the uveoscleral or nonconventional outflow pathway and the trabecular or conventional pathway, which comprises the trabecular meshwork (TM) and Schlemm's canal. The balance between the two routes is established by the ciliary muscle tone. More fluid leaves the eye via the uveoscleral pathway when the ciliary muscle is relaxed than when it is contracted. Figure extracted from *Llobet et al.* (2003).

dystrophy do not have increased rates confirming there is no compensatory mechanism to reduce aqueous flow as IOP increases (*Benos et al.*, 2008). Outflow facility does not decrease enough during sleep to compensate for the decreased AH flow rate, indicating that changes in episcleral venous pressure and/or uveoscleral flow may happen (*Sit et al.*, 2008).

3.2. Aqueous Humor Outflow

The AH exits the eye through both conventional (*trabecular outflow*) and nonconventional pathways (*uveoscleral outflow*). The uveoscleral pathway refers to the aqueous leaving the anterior chamber by diffusion through intercellular spaces among ciliary muscle fibers and represents only 10-20% of the total outflow (*Bill and Phillips*, 1971; *Pederson et al.*, 1977). The physiology of these two routes differs in several important ways; the uveoscleral outflow pathway is relatively independent of the IOP

compared to the trabecular pathway, they respond differently to pharmacological agents, and they have different limiting steps to aqueous flow (**Figure 3**).

3.2.1. Trabecular outflow (conventional pathway)

Majority of the AH exits the eye through the trabecular pathway that is IOP dependent. It is measured as *outflow facility*, and expressed in microliters per minute per millimeter of mercury (μL/min/mmHg). Although most open-angle glaucoma (OAG) are caused by an increase in the resistance to aqueous drainage through this pathway, there are still no drugs to treat glaucoma developed to target directly the TM since many aspects of its regulation still remain unclear.

On this route, AH drains from the anterior chamber through progressively smaller intercellular spaces of the TM into the SC, which drain directly into the AVs. Resistance to AH flow increases progressively from anterior chamber to SC as intercellular spaces narrow, reaching the highest at the cribiform or juxtacanallicular TM together with the inner wall of the SC. In humans, 75% of this resistance is localized within the TM, while 25% occurs beyond the SC (*Grant*, 1958). Aqueous then crosses the inner wall endothelium of the SC by two different mechanisms: a paracellular route through the junctions formed between the endothelial cells and a transcellular route through intracellular pores of the same cells. Intracellular pores are associated with the formation of cellular outpouchings or giant vacuoles when the AH pushes against the basal side of the inner-wall endothelium. The formation of these vacuoles is pressure dependent but gradual increase in IOP leads to progressive collapse of the canal, increase of outflow resistance and further IOP rise (*Goel et al*., 2010; *Gong & Francis*, 2014).

AH outflow is non-uniform or “segmental” pattern since it only occurs in a certain fraction of the outflow pathways potentially available in the 360° at a given time. This active area is termed the *effective filtration area* (EFA) and can be identified by studying the distribution of pigment in the TM and tracer perfused into the anterior chamber. Greater concentration of tracer and more pigmentation in the TM near the CC ostia serve to identify these regions with active flow (*Gong & Francis*, 2014).

Accumulation of extracellular matrix in the juxtacanallicular TM, collapse of SC, increasing herniations of the inner wall and juxtacanalicular tissue into the CC ostia and blockage of the CC would reduce the EFA and outflow facility. All these morphological changes have been observed in patients with primary open angle glaucoma (POAG) (*Gong & Francis*, 2014).

3.2.2. Uveoscleral outflow (nonconventional pathway)

Through the uveoscleral pathway the AH enters the ciliary muscle and exits the eye by multiple routes. It can flow across the anterior sclera within supraciliary and suprachoroidal spaces and across the posterior sclera. It can cross through the emissarial canals around the vortex veins, or into the choroidal vessels. Fluid also enters the ciliary processes from where is secreted back into the posterior chamber (*Benos et al.*, 2008).

The uvesocleral outflow (Fu) has been considered an analogous to lymphatic system, as it has long been accepted the eye has no lymph vessels. Recently though, using specific immunostaining for lymphatic vessels, numerous fine channels in the ciliary body and conjunctiva suggestive of an uveolymphatic pathway in the eye (*Yücel et al.*, 2009).

Although this pathway is considered independent of IOP, as observed in monkeys, the relationship between flow and IOP is not linear (*Bill*, 2003). The uveoscleral outflow is greatly affected by the contraction of the ciliary muscle that increases the flow resistance of its intersticial spaces (*Goel et al.*, 2010).

The uveoscleral outflow cannot be measured directly with non-invasive methods, thus requiring a mathematical calculation, the *Goldmann equation*. Uveoscleral drainage can account from 4% to 54% of total aqueous flow in humans (*Alm & Nilsson*, 2009) It tends to decrease with age (*Toris. et al.*, 1999b), thus the trabecular outflow must compensate the effect of aging to prevent an increase in IOP. It is not known if uveoscleral flow in human eyes with POAG is reduced. In healthy adults between 20-30 years of age Fu has been estimated to be around 1,52 and around 1,10 μl/min (T*oris. et al.*, 1999b). Clinical studies have been made in eyes with ocular hypertension (OHT)

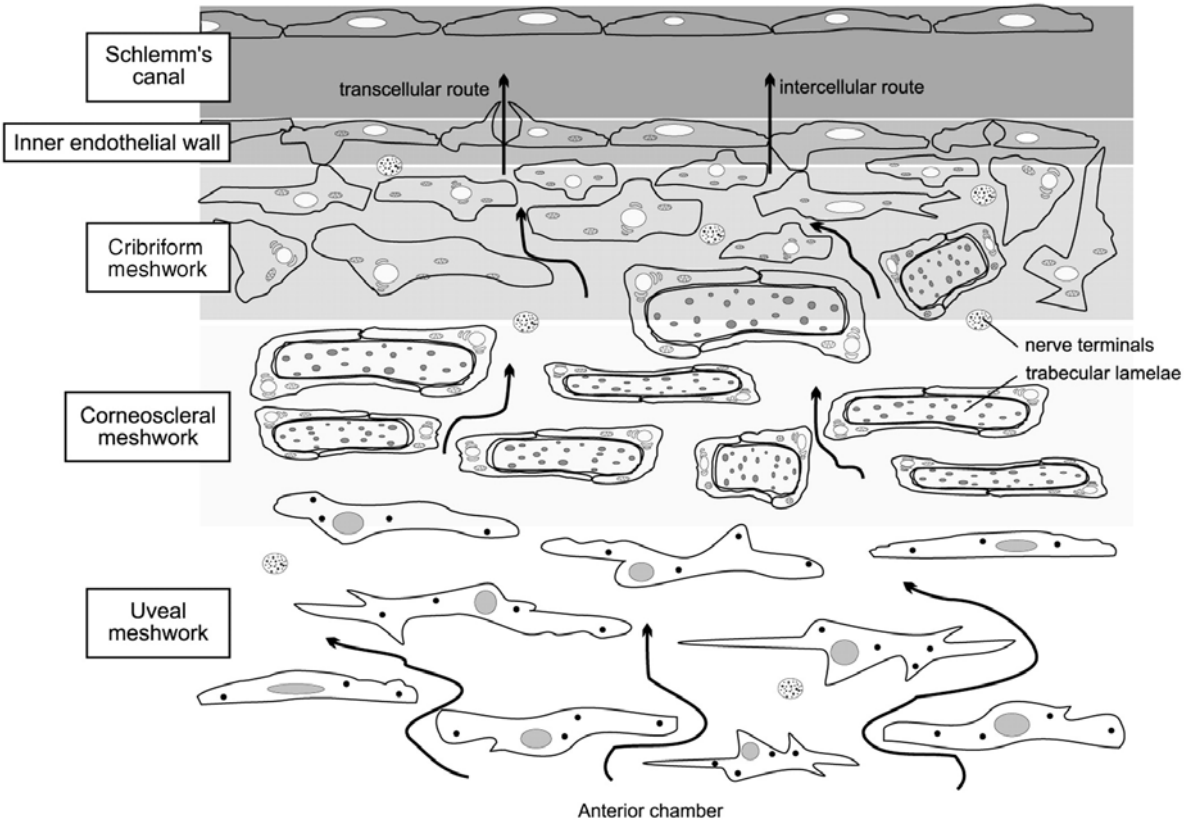


Figure 3. Schematic diagram of the TM. The arrows indicate the direction of the aqueous humor, from the anterior chamber toward Schlemm's canal. The different regions of the TM are the uveal meshwork, corneoscleral meshwork, juxtacanalicular or cribriform meshwork, inner wall of Schlemm's canal, and Schlemm's canal. Aqueous humor flows through the intercellular spaces of the TM and crosses the inner wall of Schlemm's canal via two different mechanisms: an intercellular route and a transcellular route. Resistance to aqueous humor flow increases progressively from the anterior chamber to Schlemm's canal as intercellular spaces narrow. Figure extracted from *Llobet et al.* (2003)

with inconclusive results (T*oris et al.*, 2002; *Thomas et al.*, 2008). It has been found to be markedely reduced in eyes with pseudoexfoliation. (*Jhonson et al.*, 2008)

3.3. Intraocular pressure: the Goldmann equation

The IOP describes the tension exerted by the AH in the intraocular tissues as a result of the balance between its production and drainage. IOP is dynamic and determined by several variables. The relationship between the IOP and these variables can be modeled by the modified *Goldmann equation*:

$$IOP = Pv + (Fin - Fu)/C$$

where, *Pv* is the episcleral vein pressure, *Fin* is the aqueous flow, *Fu* is the outflow through the uveoscleral pathway and *C* the trabecular outflow facility.

In physiological conditions there should be a balance between the production and evacuation of the AH from the eye,

$$Fin = Fout = F\,trab + Fu$$

where, Fin is the inflow of aqueous humor into the eye; Fout is the evacuation of aqueous humor from the anterior chamber; Ftrab is the outflow through the trabecular pathway and Fu the outflow through the uveoescleral pathway.

Ohm's law states that current (*I)* equals the voltage difference *(ΔV)* divided by resistance *(R)*. If we apply Ohm's law to hemodynamics, the flow across a given system *(Q)* is directly proportional to the pressure difference *(ΔP)* between any two points along this system and inversely proportional to its resistance *(R)*.

$$\Delta P = Q / R$$

This equation can be applied to the fluid dynamics of the eyeball considering the evacuation of aqueous through the trabecular is dependent on IOP while the uveoscleral drainage is pressure-independent. The force that facilitates the drainage through the trabecular meshwork is the hydrostatic pressure difference between the anterior chamber and the episcleral veins. Thus, we could apply the Ohm's law to the fluid dynamics of the eye as:

$$Ftrab = (Fin - Fu) = (IOP - Pv) / R$$

Where *Pv* is the episcleral vein pressure, *IOP* is the intraocular pressure and *R* the resistance through the trabecular or conventional route. If the resistance is expressed as outflow facility *(C)*, which is the inverse of resistance, the formation of aqueous can be expressed as:

$$Fin = (IOP - Pv) \times C + Fu$$

This formula that describes the aqueous dynamics of the eye is known as *Goldmann equation (modified)*. Not until the uveoscleral pathway was discovered (*Bill*, 1965) and uveoscleral outflow (Fu) included in the formula, investigators were able to balance the equation (*Brubaker*, 2004). All aqueous dynamics parameters except of uveoscleral outflow can be measured by non-invasive methods. Nevertheless, to calculate accurately the uveoscleral outflow, three criteria should be met. First, the measurements of all other parameters must be accurate. Second, the pressure-dependancy of outflow into the episcleral veins must remain constant and linear for the range of IOP where C is measured and where the equation is to be applied. Third, uveoscleral outflow must be pressure independent over the same range of IOP (*Brubaker*, 2004).

IOP in the healthy population is normally distributed with a mean of 15-16 mmHg and a standard deviation of 2.6 mmHg (*Leydhecker et al.*, 1958). This led to the definition of "normal" IOP as 2 standard deviations above and below the mean IOP, or approximately 10–21 mm Hg. Although the IOP follows a normal distribution, it is skewed towards higher pressures, so IOPs >21mmHg would thus not necessarily represent abnormality from a statistical standpoint.

In humans, the IOP is usually measured by applanation tonometry, being the *Goldmann applanation tonometer* the most commonly used in a clinical setting.

4. Aqueous Humor dynamics: Study Methods

4.1 Intraocular Pressure: Tonometry

Most comercially available tonometers estimate IOP by relating a deformation of the cornea to the force responsible for that deformation. Depending on how the eyeball is deformed tonometers are classified in applanation (flattening) or indentation tonometers.

4.1.1. Indentation Tonometers

The force can be applied by digital pressure or a known weight. The prototype of this type of tonometer is the *Schiötz tonometer* (**Figure 4**). The shape of the deformation

with this type of tonometer is a truncated cone. The indentation will be greater if the internal pressure is low than when the internal pressure is high. The tonometer is allowed to rest on the patient's cornea, and the extent to which the weighted plunger of the tonometer idents the cornea is shown on a scale by a simple lever arm indicator. The lever arm system magnifies the motion of the plunger 20-fold so that a 0.05 mm movement of the plunger is represented by a 1 mm space between units on the tonometer scale. When the weighted plunger of the Schiötz tonometer indents la cornea, the baseline or resting pressure (P_0) is artificially raised to a new value (P_t).

The change in pressure from P_0 to P_t is an expression of the resistance an eye offers to the displacement of a volume of fluid. Conversion tables based on empirical data from in vitro and in vivo studies must be used to translate scale readings into estimates of IOP (P_0) (*Alguire*, 1990; *Allingham et al.*, 2011). The accuracy of the IOP estimation is limited by the oscillation of the indicator needle caused by the ocular pulse and by a physiologic moment-to-moment variations in IOP, variability of the elastic properties of the eye and by the curvature of the cornea.

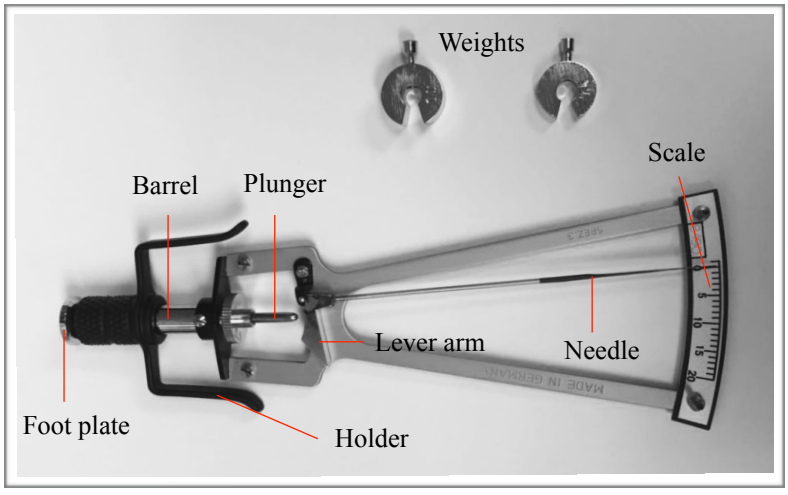


Figure 4. The Schiötz tonometer. It consists of a hollow barrel with a concave footplate and a holder. A free-floating, rod-like plunger with a 5.5 gram weight attached fits inside the barrel.

4.1.2. Applanation tonometers

Applanation tonometry is based on the Imbert-Fick principle that states "the pressure (F) in a sphere filled with fluid and surrounded by an infinitely thin and flexible membrane is measured by the counter-pressure (P) which just flattens the membrane to a plane" (*Mark*, 2012; *Markiewitz*, 1960). This so-called "law" expressed as $F = P \cdot A$, where A is the area flattened by the applied force, assumes such membrane is (a) perfectly spherical, (b) dry, (c) perfectly flexible, and (d) infinitely thin. However, the

hypothetical membrane does not fit the corneal model. Thus, the accuracy of the IOP measurements depends on individual corneal properties such as corneal thickness, curvature and biochemical properties (*Moraes et al.*, 2008)

In 1954 H. Goldmann modernised the earlier tonometers and invented the *Goldmann applanation tonometer* (GAT) (*Goldmann*, 1954) that has become the gold standard against which all the others are judged. The GAT measures the force necessary to flatten an area of the cornea of 3,06 mm diameter. He adopted and modified the Imbert-Fick law to $F+S = Pt \cdot A_1 + B$, where S is the surface tension, A_1 is the inner area of the flattening and B refers to ocular rigidity. When A_1 equals 7.35 mm^2, S balances B, and F, equals Pt. This internal area of applanation is obtained when the diameter of the external area of corneal applanation is 3.06 mm, diameter of the cylinder used in the standard instrument. The volume of displacement produced by applanating an area with a diameter of 3.06 mm is approximately 0.50 mm^3, so that P_t is very close to P_0, and ocular rigidity does not significantly influence the measurement. These calculations were based on an average central corneal thickness (CCT) of 500 - 525 μm, therefore deviation from the average can affect the measurements. Increased CCT may give artificially high values whereas decreased CCT may give low values (*Allingham et al.*, 2011).

4.1.3. Applanation-Identation tonometers

The *pneumatic tonometer or pneumotonometer* (PT, Model 30 Classic; Reichert Ophthalmic Instruments, Depew, NY) contrary to GAT can measure the IOP in any body position, does not require topical application of fluorescein and has optional tonography functions. It measures IOP by calculating the force required to flatten an area of cornea using an elastic membrane inflated with air. It consists of a 5 mm-diameter silicone membrane fixed to a light plastic tip attached to a plastic piston that rides on a nearly frictionless “air bearing”. The plunger is driven against the cornea by a regulated flow of air. The air pressure increases until the cornea and membrane on the tip of the plunger are flat. At this time, the pressure applied on the cornea equals the pressure in the anterior chamber and the IOP is recorded. Although the pneumatic tonometer was designed as an applanation device, it may display some of the properties of an

Figure 5. Pneumatic tonometer or pneumotonometer used in this study to measure the IOP without fluorescein. (Model 30 Classic; Reichert Ophthalmic Instruments, Depew, NY).

indentation tonometer by deforming the cornea and displacing a significant amount of intraocular fluid.

There are not many studies comparing GAT and PT, but PT seems to overestimate IOP (*Barkana & Gutfreund*, 2014; *West et al.*, 1972). In eyes with previous laser-assisted in situ keratomileusis (LASIK) surgery, mean IOP measured with GAT was found to be 3.8 mmHg lower than with PT. In the same study a significant positive correlation between CCT and IOP measured with GAT was found but not with PT (*Bayraktar & Bayraktar*, 2005).

In this study we used a PT to measure the IOP since the application of topical fluorescein required to measure the IOP with GAT would have interfered in the acquisition of the fluorophotometric scans.

4.2 Aqueous Humor Production: Fluorophotometry

Fluorophotometry uses non-invasive optical methods to determine the concentration of fluorescein in the ocular tissues and compartments. Is the most commonly used technique to measure the production of aqueous humor in vivo.

Fluorescence is the property of some atoms and molecules to absorb light at a particular wavelength and to subsequently emit light of longer wavelength after a brief interval. In the visible light spectrum, that translates into a change of color.

Over the years, several investigators engineered different techniques to study the production of aqueous humor. However, initially, these methods were too invasive to study the human eye *in vivo. A*t the end of the 19th century, Ehrlich demonstrated that

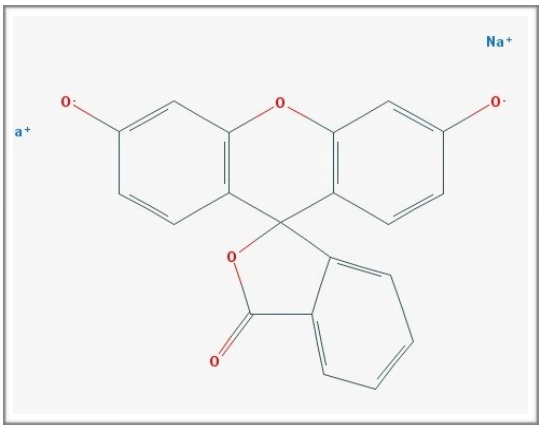


Figure 6. Fluorescein sodium 2D structure (**C_{20} H_{10} Na_2 O_5**)**.** Figure extracted from https://pubchem.ncbi.nlm.nih.gov.

fluorescein administered systemically to a rabbit appeared in the aqueous humor. In 1946, Amsler and Huber designed a method to study fluorescein's ocular permeability. They estimated the concentration of fluorescein, using a slit lamp equipped with an ammeter and rheostat, by measuring the intensity of the current through the lamp filament that progressively diminished until the fluorescence had disappeared (*Brubaker*, 1982). In 1950, Goldmann, developed the first mathematical model of intravenous fluorescein kinetics. Langley and MacDonald were the first to introduce fluorescein into the eye by topical administration. Langham and Wybar built the first objective slit lamp fluorophotometer using a photomultiplier tube that measured the intensity of fluorescence in the eye. However, it was not until 1963 that Maurice built the first fluorophotometer as we know it nowadays, capable of quantifying the fluorescence contained in the ocular structures. Together with Jones, in 1966, they developed a mathematical method to study the aqueous humor dynamics. The fluorescein was introduced into the eye through the cornea by an electric current or iontophoresis. They were able to measure the fluorescein clearance from the anterior chamber and, consequently, the aqueous humor flow through the anterior chamber (*Brubaker*, 1982).

Fluorescein sodium (C_{20} H_{10} Na_2 O_5). It is a xanthene dye synthesised by Adolf von Baeyer in 1871 (**Figure 6**) (*Baeyer*, 1871). It has an orange-red color and a molecular weight of 376.27 g. Its most important property is *fluorescence.* Fluorescein sodium absorbs blue light, with peak excitation occurring at wavelengths between 465-490 nm. The resulting fluorescence occurs at the yellow-green wavelengths of 520 to 530 nm. Dye concentration and pH can affect the intensity of fluorescence. Maximum fluorescence occurs at a pH of 7,4.

Many of the properties of fluorescein make it an ideal tracer for use in ophthalmic research. Of greatest importance are the fluorescent efficiency of fluorescein, the lack of variation in this efficiency at physiologic pH, the lack of oxygen quenching at normal atmospheric pressures, and the lack of photo-degradation of fluorescence (*Brubaker*, 1982).

The fluorescence is proportional to the fluorescein concentration in the dissolution, although at concentrations higher than 10^{-5} moles/L, the progression is not strictly linial.

Fluorescein is non-toxic and it can be administrated topically, orally or intravenously. When administered intravenously, fluorescein is bound loosely to albumin. In human plasma, the unbound concentration of fluorescein is approximately 15% of the total, and this fraction is constant for fluorescein concentrations lower than 10^{-4} mols/L (*Brubaker et al.*, 1982). After being administered, the fluorescein converts quickly to fluorescein glucoronide that also fluoresces, and within 10 min the concentration of unbound fluorescein glucuronide exceeded that of unbound fluorescein. The terminal half-lives of fluorescein and fluorescein glucuronide in the plasma ultrafiltrate are 23.5 and 264 min, respectively, so that fluorescein glucuronide contributes almost all of the plasma fluorescence after 4-5 hr (*Blair et al.*, 1986). Fluorescein and its metabolites are mainly eliminated via renal excretion. After IV administration, the urine remains slightly fluorescent for 24 to 36 hours. The fluorescein can also be instilled topically. *Fluorescein 2%* (2 x 10-2 g/mL) administered in the conjunctival cul-de-sac penetrates the corneal epithelium and stroma where it creates a reservoir. Having penetrated the epithelium and entered the stroma, fluorescein, which is not metabolised in the eye, can disappear in one of three ways: (1) by rediffusing through the corneal epithelium and flowing away with the tears, (2) by diffusing laterally into limbal tissue, and (3) by penetrating the endothelium and entering the aqueous humor. The third pathway offers the least resistance and consequently is the major route of loss from the stroma. Once in the anterior chamber, the tracer is washed away by flowing aqueous or diffuses into the iris. This diffusional loss has been shown to account for less than 10% of the total clearance. A waiting

period of 6 hr or more allows the dye to become distributed uniformly in the stroma (*Brubaker,* 1991).

All methods to study the production of the aqueous humor are based on the same principle: the fluorescein depot created in the anterior chamber is slowly removed. The speed at which the fluorescein disappears from the anterior chamber is an estimation of the aqueous flow rate. This technique permits to study the production of aqueous humour in humans in different conditions, pathologies and under the effects of different pharmacological agents.

Figure 7. The FM-2 Fluorotron Master used in our laboratory.

4.2.2. Fluorotron Master

Fluorophotometry emits a specifically focused excitation beam of blue light into the ocular cavity creating a receiving beam of fluorescent green light that is detected by a photodetector. Only fluorescence at the intersection of the two beams is measured. The primitive fluorophotometers were coupled to a slit lamp, but modern devices use an optical head with an automatic scanning system along the optical axis of the eye. The collected light is passed through a filter that selects radiation near 500 nm and finally quantifies the number of photons through a photomultiplier tube.

The **FM-2 Fluorotron Master** (FM, Ocumetrics, Inc.) is a commercially available ocular fluorophotometer that consists of four major components; the optic head, computer, display monitor, and printer. The fluorophotometer can change the

focal plane every 0.25 mm, thus allowing up to 149 sequential readings from the anterior cornea to the posterior retina. The results are processed by the computer, and the monitor can be observed by the investigator. The optical head is designed to allow easy alignment with the patient and obtain a scan by pressing a single button. The scan is displayed graphically and a hard copy can be obtained of both the graphic and the numerical data. Scan and patient data are stored on a magnetic fixed disk. The computer is used to process the data using the appropriate software. **Figure 8** is a schematic representation of the optical system of the FM (*Munnerlyn et al.*, 1985). Light from the tungsten halogen source passes through the blue exciter filter and the narrow source slit. A folding mirror directs the light through the outer portion of the fixed collimating lens. The collimated beam passes through the outer position of the scanning lens. This lens forms an image at the focus of the eyepiece, which is half the size of the source slit. The beam fills the outer portion of the eyepiece lens and is collimated prior entering the eye. If the eye is ametropic, the excitation light will come to focus at the retina. Any fluorescent material along the patch will produce a fluorescent glow within the beam. The detection path is controlled by an aperture on the opposite side of the fixed collimating lens. This optical path is separated from the excitation beam at all points except for a diamond-shaped overlap region within an eye and at the focus of the scanning lens. Only fluorescent light originating from within this diamond-shaped reaches the photomultiplier. The fluorescent ligh travels back through the eyepiece, the scanning lens, the collimating lens, the collimated lens aperture, and the detection split. The detection split coincides optically with the source split. After passing through the detector split, it passes through the barrier filter which blocks any blue light due to scatter or reflection within the eye. The photo-multiplier cathode collects the light and converts it to electrical impulses, which are counted by the computer system. To be able to measure the aqueous flow rate an anterior chamber adapter is needed. A lens attachment is used to increase the convergence angle at the cornea (*Munnerlyn et al.*, 1985). **Table 1** describes the characteristics of the device.

Table 1. FM-2 Fluorotron Master specifications provided by Ocumetrics, Inc.

Optic Head	
Depth of Resolution	2 mm at 3% peak signal
Sensitivity	0.1 ng / mL fluorescein (3 x background fluctuations)
Reproducibility	3% with solutions > 5 ng / mL 5% with solutions < 5 ng / mL

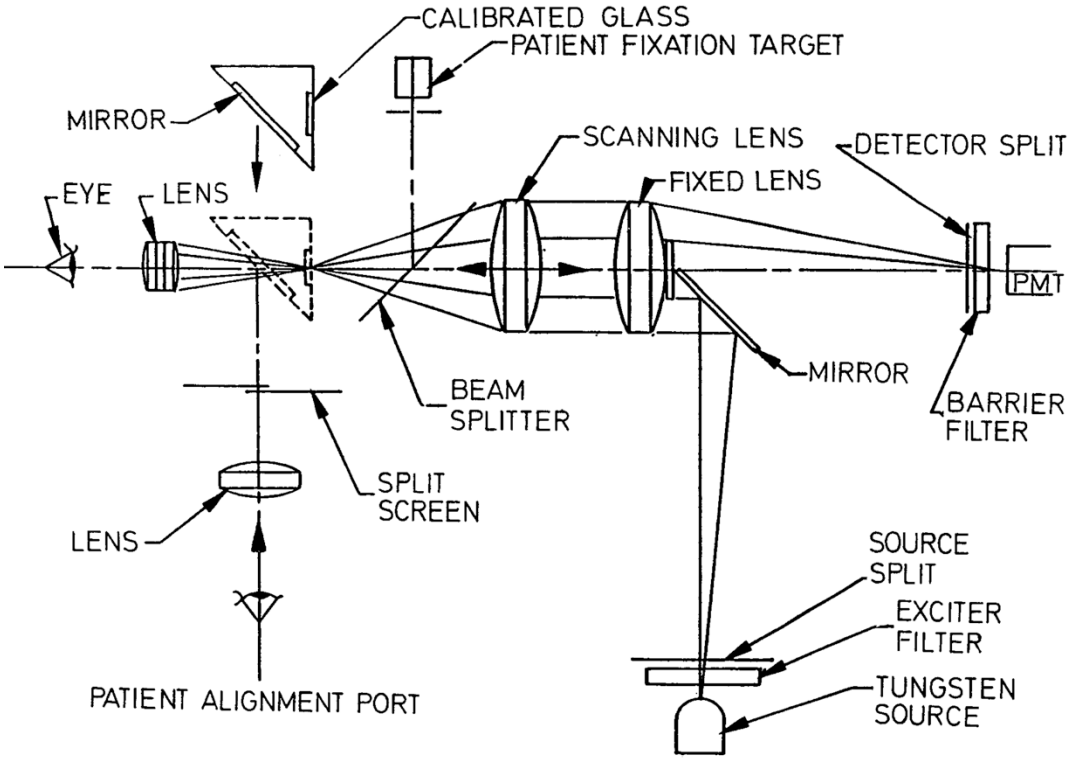


Figure 8. Optical schematic of Fluorotron Master. Extracted from *Munnerlyn et al.* (1985).

The FM was originally designed to measure the leakage of fluorescein dye from the retina into the vitreous in a similar fashion that fluorescein angiography photographs this leakage. It can measure the blood-retinal barrier permeability, vitreous diffusion, lens autofluorescence, blood-aqueous barrier, aqueous turn-over, cornea endothelial permeability, cornea epithelial permeability and tear turn-over.

Crystalline lens and cornea fluoresce naturally. The lens has an autofluorescence higher tan 200 ng/mL (*Beneyto & Perez*, 2006), that increases with lens aging. As a person ages, glucose reacts with the proteins in the lens to produce new compounds that fluoresce. Contrary to the lens, cornea autofluorescence is not age-related. The cornea shows a peak of $20x10^{-9}$ g/mL (*Kitaya et al.*, 1998), that corresponds to the corneal stroma. The fluorescence of the tear film is undistinguishable from the corneal peak, but in vitro it does not reach values higher than 0.2 ng/mL. Aqueous humor and vitreous have an autofluorescence close to zero.

Aqueous turnover can be determined by staining the cornea with fluorescein eye drops and monitoring its movement from cornea to anterior chamber and out. The FM-2 software uses the **Yablonski protocol** to calculate the aqueous flow rate (*Yablonski*, 1978). The fluorophotometer measures the concentration of fluorescein in the corneal stroma and anterior chamber at the beginning and at the end of a time interval. The production of aqueous humor equals the clearance of fluorescein during this interval minus the diffusional loss through the iris. Thus, measuring the fluorescein concentration in the corneal stroma and anterior chamber at different time intervals, fluorescein clearance rate and indirectly, aqueous production rate, can be calculated. During the first 2 to 3 hours after the instillation of fluorescein eye drops, the concentration of fluorescein in the cornea falls as the concentration in the anterior chamber rises. After that initial period, both concentrations fall in a very consistent manner.

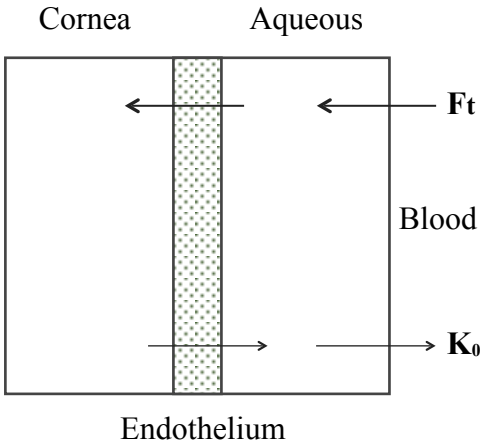


Figure 9. Bicompartimental model. The pharmacodynamics of the topical fluorescein can be studied using a bicompartimental model (corneal stroma and anterior chamber). The fluorescein which is slowly released from the corneal stroma to the anterior chamber, escapes together with the aqueous humor through a constant inflow (Ft) and outflow of aqueous (K_o).

The program calculates the aqueous turnover directly from the equation:

$$K_O = A\left[1 + \frac{VcCc}{VaCa}\right]$$

where,

$$A = \frac{d\ln(Cc)}{dt} = \frac{d\ln(Ca)}{dt}$$

The program uses the average of these two concentration decay rates as computed using least squares linear regression.

Vc = volume of the cornea (µL)
Va = volume of the anterior chamber (µL)
Cc = cornea fluorescein concentration (ng/mL)
Ca = anterior chamber fluorescein concentration (ng/mL)

The ratio of corneal fluorescein concentration versus anterior chamber fluorescein concentration at the mid-experiment time point is used as predicted by the best fit equations of the data determined by least squares linear regression. Aqueous flow is computed as:

$$Ft = Ko \cdot Va$$

where, Ft is the aqueous humor flow (µL/min), Va is the volume of the anterior chamber (µL), and Ko is the loss coefficient due to bulk flow and diffusion from the anterior chamber (minutes^{-1}). Ko can be thought of as the fraction of anterior chamber volume cleared of fluorescein every minute, due to aqueous flow.

4.3. Aqueous humor outflow: Tonography

The amount of resistance or inversely, the facility of outflow (C) can be measured using tonography.

4.3.1. Indentation Tonography

The term **tonography** describes very well this method of investigation that consists of a recording of the IOP changes that take place when a weight is held onto the

cornea for a measured period of time. A weighted tonometer e.g. electronic Schiötz tonometer or pneumatonometer, is placed on the patient's cornea for 2-4 minutes. The IOP initially increases, causing the rate of aqueous outflow increase while the IOP slowly falls toward the initial pressure (*Grant*, 1951). The rate at which the IOP decreases with time is related to the ease at which the aqueous leaves the eye, being able to calculate the outflow facility.

Unfortunately, indentation tonography is subject to a number of *sources of errors* that can affect the accuracy and reproducibility of the measurements. It assumes that a steady-state exists in the eye before tonography begins. It is assumed that inflow and outflow are equal, that facility of outflow remains unchanged and that episcleral venous pressure and ocular blood volume remain constant and unaltered once the tonometer is placed on the cornea; that the eye expands and contracts according to a known equation not compensating individual variations in ocular rigidity; and that the volume displacement is exclusively due to tonometry rather than the examiner or eyelid squeezing (*Brubaker*, 2004). Tonography assessed with a pneumatonograph is less affected by ocular rigidity than the Schiötz tonography, because the probe that is placed on the eye creates a relatively smaller corneal indentation.

Once the electronic Schiötz tonometer has been calibrated, with the patient comfortably recumbent, the plunger is allowed to rest on the patient’s topically anesthesized cornea for at least 4 minutes. During this time, the tonometer and recorder will show the IOP has been gradually falling. The aim is to obtain a smooth tonographic curve with superimposed ocular pulse. Before the tonometer is applied, the eye is in a steady state, with a steady IOP, steady rate of aqueous production and outflow. When the tonometer is placed on the eye the IOP is immediately artificially raised, as a result of indentation of the cornea. Raising IOP raises the rate of outflow of aqueous, with little or no effect on the aqueous production. When the outflow exceeds the inflow, the IOP begins to fall, gradually approaching the pressure of the original steady state. The rate of fall depends on the facility of aqueous and the height to which the IOP is raised above the steady state.

The C (μL/min/mmHg) value may be found to range from as little as near zero to as high as 0.50. Patients with a C below 0.18 may be considered in the glaucomatous range, especially those with a C <0.13 (*Garner,* 1965).

Outflow facility can be also measured by pharmacologically suppressing the rate of aqueous humor formation using acetazolamide, dorzolamide or timolol, and measuring the drug-induced change in IOP by tonometry, and the change in aqueous flow by fluorophotometry (Hayashi *et al.*, 1989). Brimonidine and apraclonidine are not appropriate for this purpose, because they also affect outflow (*Toris et al.*, 1995, 1999a).

4.4. Episcleral venous pressure:

The AH leaves the SC through collector channels, aqueous veins and episcleral vessels. The episcleral venous pressure (EVP) can be described as a back-pressure against which aqueous humor must flow. The pressure in the episcleral veins averages about 7–11 mmHg in humans (*Sit & McLaren*, 2011).

Ageing does not seem to affect significantly EVP (*Toris et al.*, 1999b) but it is markedly influenced by posture, increasing in supine position (*Friberg et al.*, 1987; *Sultan & Blondeau*, 2003).

The change in EVP occurs rapidly within 1 min and is associated with a rapid increase in IOP. It is estimated that IOP increases by 0.83±0.21mmHg for every increase of 1 mm Hg in episcleral vein pressure (*Friberg et al.*, 1987). Chronic elevations of EVP may result in more complex changes in IOP. Abnormal elevated EVP can cause the collapse of SC and an increase in the aqueous humor resistance. Increased EVP is known to be a cause of glaucomatous optic neuropathy, e.g., in association with carotid cavernous fistula, cavernous thrombosis, Graves disease or Sturge-Weber syndrome.

An accurate assessment of EVP is necessary to estimate the uveoscleral outflow. Several techniques have been described for the measurement of the episcleral venous pressure, but no completely satisfactory method exists. It can be measured by invasive and non-invasive methods. *Invasive measurements* such as direct cannulation of the episcleral veins and the intracameral micro-needle method (to control the pressure in

the anterior chamber) are more accurate, but cannot be used in humans (*Benos et al.*, 2008; *Sit and McLaren*, 2011). *Non-invasive measurement* of EVP is based on the principle of venous compression. The venous pressure is determined from the force applied to a given episcleral vein necessary to collapse such vessel to a predetermined endpoint. The *pressure chamber technique* is the most common method to measure the EVP non-invasively. It uses a clear and flexible membrane attached to a pressure chamber to apply a force sufficient to collapse an episcleral vein. The *Episcleral Venomanometer* is the only commercially available device (Model EV-310, EyeTech Ltd, Morton Grove, IL). The venomanometer is attached to a slit-lamp and the subject is positioned such that the episcleral veins near the limbus can be seen through the binoculars. It consists of a hollow applanating head filled with air with a clear overlying membrane. The pressure within the head is increased until the underlying episcleral vein collapses. The vein collapses gradually as the pressure increases. It seems the critical measurement endpoint of collapse is between baseline, when the pressure in the chamber is equal to atmospheric pressure, and total collapse of the vein (*Sit and McLaren*, 2011). The venomanometer requires the endpoint to be determined by direct observation. This subjective judgement of the endpoint makes the EVP measurements variable and uncertain. This technique has not gained any ground as a clinical or research tool due to lack of precision and reproducibility. Recently, a new method based on the pressure chamber technique to objectively identify the compression endpoints of the vessels has been developed (*Sit et al.*, 2011). It is a computerized system able to synchronise the images, captured by a high-definition video camera, of the vein as it is compressed and the pressures applied to inflate the membrane at a constant rate for a preset duration and pressure in the chamber.

5. Aqueous Humor Dynamics Alterations

5.1. Ocular Hypertension, Primary Angle Glaucoma and Ethnicity

Primary open angle glaucoma (POAG) is a chronic progressive neuropathy with characteristic morphological changes at the optic nerve head and retinal nerve fibre layer, that leads to progressive visual field loss, in the absence of other ocular disease or congenital anomalies. Glaucoma is the second leading cause of blindness and the most frequent cause of irreversible blindness worldwide (**Figure 10**, *Tham et al.*,

2014). A recent meta-analysis of 53 population-based glaucoma studies demonstrated the variation in prevalence of glaucoma across geographic regions and ethnic groups (*Tham et al.*, 2014). They estimated the global prevalence of glaucoma for population aged 40-80 years to be 3.54%, with an estimated prevalence of POAG of 3.05% and that of *primary angle closure glaucoma* (PACG) of 0.5%. The number of people with glaucoma worldwide is estimated to increase from 64.3 million in 2013 to 76.0 million in 2020, and to 111.8 million in 2040. In the Bayesian meta-regression model, after adjusting for age, gender, habitation type, response rate, and year of study, people of African ancestry were more likely to have POAG than people of European ancestry (**Table 2**, *Tham et al.*, 2014; *see page 36*).

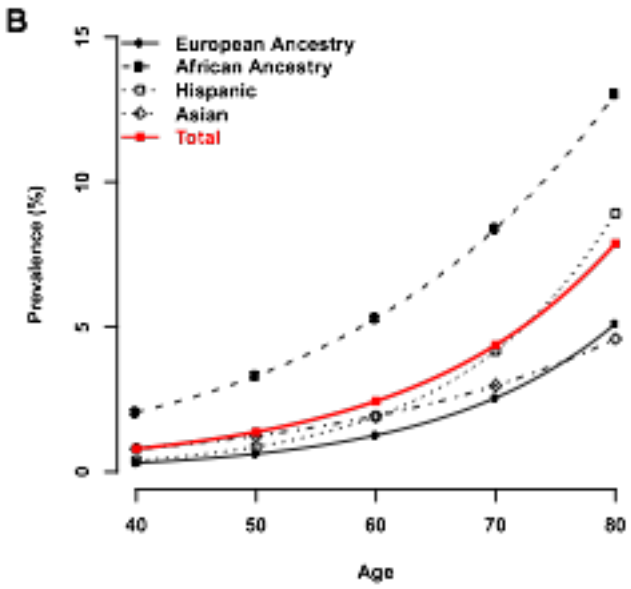


Figure 10. Age-specific prevalence of primary open-angle glaucoma (POAG) by ethnic groups. Figure extracted from *Tham et al.* (2014)

Table 3 (*see page 37*) shows the prevalence of OAG in people of African descent from different surveys (*Budenz et al.*, 2013).

*Ocular Hypertension (*OHT) is by definition a condition characterised by elevated IOP in the absence of identifiable optic disc damage or visual field loss. It is estimated that 3 to 6 million people in the United States, including 4% to 7% of those older than 40 years, have elevated IOP without detectable glaucomatous damage on standard clinical tests (*Leibowitz et al.*, 1980). In the United States, the prevalence of OHT in non-Hispanic whites who are 40 years of age and older is 4.5%, and increases up to 7.7% in 75 to 79 year olds. In Latinos, the prevalence across ages is quite

similar. Because OHT occurs typically without symptoms, a majority of people with OHT remains undiagnosed. In the Los Angeles Latino Eye Study, 75% of Latinos with IOP greater than 21 mmHg were previously undiagnosed (*Varma et al.*, 2004).

Table 2. Demographic factors associated with Primary Open Angle Glaucoma.

	Odds ratio(95% Crl)*	
	Age and gender adjusted	**Multiple adjusted**
Age, per decade increase	1.75 (1.65-1.84)	1.73 (1.63-1.82)
Gender		
Women	1.0 [Reference]	1.0 [Reference]
Men	1.36 (1.23-1.51)	1.36 (1.23-1.52)
Geographic region		
Asia	1.0 [Reference]	1.0 [Reference]
Africa	2.39 (1.17-4.53)	1.97 (0.92-3.72)
Europe	0.87 (0.56-1.32)	0.69 (0.35-1.18)
North America	1.36 (0.66-2.56)	1.23 (0.53-2.50)
Latin America and the Caribbean	2.21 (0.96-4.56)	1.53 (0.52-3.41)
Oceania	0.98 (0.41-1.97)	0.83 (0.30-1.92)
Urban/rural		
Urban	1.0 [Reference]	1.0 [Reference]
Rural	1.51 (1.17-1.90)	1.58 (1.19-2.04)
Mixed	2.18 (0.55-5.77)	1.90 (0.47-5.44)
Ethnicity		
European ancestry	1.0 [Reference]	1.0 [Reference]
African ancestry	2.88 (1.97-4.10)	2.80 (1.83-4.06)
Hispanic	1.28 (0.44-3.14)	2.00 (0.57-5.15)
Asian	1.12 (0.77-1.55)	1.43 (0.82-2.34)

CrI = credible interval.

*Calculated on the basis of Bayesian meta-regression model.

#Adjusted for age, gender, habitation type, response rate, and year of study conducted accordingly.

Table adapted from *Tham et al.* (2014).

The Ocular Hypertension Treatment Study (OHTS) included 1636 patients, aged between 40 to 80 years, with no evidence of glaucoma and with an IOP between 24 and 32 mmHg in one eye and 21 to 32 mmHg in the other and randomised patients to observation or the reduction of IOP by topical medications. The goal in the medication group was to reduce the IOP by 20% or more and to reach an IOP of 24 mmHg or less. The study reported that reducing IOP with topical antiglaucoma medication delayed or prevented the onset of POAG in individuals with OHT; at 60 months, the cumulative probability of developing POAG was 4.4% in the medication group and 9.5% in the

Table 3. Total Prevalence of Open-angle Glaucoma in People of African Descent

	Total Prevalence, percentage(95% CI)	
Source	**Observed**	**Adjusted**
Tema Eye Survey, 2006-2008	6.1 (5.5–6.8)	8.0 (7.4–8.6)
St Lucia, 1986-1987	10.2 (8.5–12.0)	9.6 (8.0–11.2)
Barbados (black and mixed), 1988-1992	6.8 (6.1–7.7)	7.4 (6.7–8.1)
Caribbean total		7.8
Baltimore Eye Survey	4.2 (3.0–5.0)	4.4 (3.6–5.2)
East Africa, 1996	3.1 (2.5–3.8)	3.6
South Africa, urban, 1998	3.7 (2.5–5.3)	3.5
South Africa, rural, 1998-1999	2.8 (1.8–4.2)	3.2

Table adapted from *Budenz et al.* (2013)

observation group, a 50% reduction risk (*Kass et al.*, 2002). After 13 years 22% of patients who had initially been randomized to the control group had converted to glaucoma compared to 16% in the treated group (*Kass et al.*, 2010). The OHTS identified elevated IOP, reduced central corneal thickness (CCT), older age, disc haemorrhages and increased cup-disc ratio as important risk factors for the development of glaucoma in patients with OHTS. The OHTS was the first randomised treatment study of OHT to recruit sufficient numbers of African American participants to examine the therapeutic benefit of ocular hypotensive medication in this group. Among African American participants in the OHTS, 17 (8.4%) of 203 in the medication group and 33 (16.1%) of 205 in the observation group developed POAG with a median follow-up of 78 months (*Higginbotham et al.*, 2004). The incidence of POAG was still significantly higher among African American participants compared with other participants in both the medication and observation groups. African American participants had twice the hazard of developing POAG in the medication group and a 58% higher hazard of developing POAG in the observation group compared with other participants. In the univariate predictive

model, self-identified African American race was associated with a 71% increase in risk of developing POAG compared with other participants. However, since African Americans participants had thinner corneas (*Brandt et al.* 2001; *La Rosa et al.*, 2001; *Shimmyo et al.*, 2003; *Friedman et al*. 2006; *Mercieca et al.*, 2007) and larger baseline cup-disc ratios than the other participants (*Quigley. et al.*, 1990; *Varma et al.*, 1994; *Zangwill et al.*, 2004), after adjusting for these factors, race was no longer associated with an increased risk of developing POAG (*Higginbotham et al.*, 2004). Progression rates of OAG have been calculated from population-based cross-sectional studies (*Broman et al.*, 2008). Although the mean progression rate was not significantly different among ethnicities (European-derived, −1.12 dB/year; Hispanic, −1.26 dB / year; African-derived, −1.33 dB /year; and Chinese −1.56 dB /year), the unilateral blindness rate due to OAG was highest in African-derived and Chinese persons, compared with European and Hispanics. These progression rates are more rapid than those reported in the Early Manifest Glaucoma Trial (EMGT, *Heijl et al.*, 2002) and Collaborative Normal Tension Glaucoma Study (CNGTS, 1998) which reported progression rate of -0.80 dB/year and -0.40 dB/year, respectively. A likely contributing factor to the greater progression and morbidity of OAG in African-derived persons is their longer average duration of disease. *Broman et al.* (2008) showed that despite having a shorter life expectancy, African-derived persons have OAG for up to 2.3 years longer than European-derived persons. This is a result of higher incidence of disease at an earlier age and probably other factors such as differential access to care and acceptance of and response to treatment.

5.2. Glaucoma treatment

The reduction of IOP has been clearly shown by several prospective randomised multi-centre controlled trials (AGIS, 2000, *Anderson*, 2003; *Higginbotham et al.*, 2004; *Kass et al.*, 2002; *Leske et al.*, 2004) to be effective in slowing down the progression of POAG as well as reducing the conversion of OHT to POAG. The IOP can be reduced using topical and oral medications, laser treatment and surgical treatment.

5.2.1. Medical treatment

Most glaucoma patients are initially treated with topical and occasionally orally administered medications that either reduce the aqueous humour production, enhance the aqueous outflow or both.

There are six classes of topical anti glaucoma drops: prostaglandin analogues, beta-receptors antagonists, carbonic anhydrase inhibitors, alpha-2 selective adrenergic agonists, non selective adrenergic agonists parasympathomimetics and osmotic agents. The following tables (**Tables 4 & 5**; *see pages 41&42*) include the most common classes of drugs and compounds and their mechanism of action.

Parasympathomimetics (cholinergic drugs)

Pilocarpine affects the outflow facility due to their effects on the ciliary muscle tone. The tendons of the ciliary muscle are partly attached to the TM and contraction of the muscle separates the trabecular lamella. An increase in conventional outflow overcomes the effect of compressing the spaces between the ciliary muscle bundles. Pilocarpine thus seems to redistribute outflow between the two outflow routes with a larger effect on trabecular than on the uveoscleral outflow (*Alm and Nilsson*, 2009).

Prostaglandin analogues

Prostaglandins seem to increase trabecular and uveoscleral outflow but varied and contradictory results regarding their mechanism of action, have been obtained by different study groups (*Alm and Nilsson*, 2009; *Brubaker*, 2001a; *Lim et al.*, 2008). Its early hypotensive effect may be mediated by a relaxation of the ciliary muscle but its long-term effect on IOP, involve a more complicated process. It is likely to be mediated by remodelling of the ciliary muscle with increased space for outflow including a reduced collagen turnover, increased metalloproteinases, changes in shape of ciliary muscle cells and alterations in the cytoskeleton proteins actin and vinculin. In support of this rearrangement of the ciliary muscle it was found that withdrawing latanoprost after 12 months treatment caused a very slow recovery of the IOP with a small but significant effect remaining after 2 weeks (*Linden et al.*, 1997).

Beta-receptor antagonists

It has been shown in several studies that systemically and topically administered beta-adrenergic antagonists lower the IOP by suppressing aqueous humor production by 30–47% (*Coakes & Brubaker.*, 1978; *Schenker. et al.*, 1981; *Yablonski et al.*, 1978) At night time during sleep, when aqueous flow is low, beta-adrenergic antagonists have no measurable effect on flow rate (*McCannel et al.*, 1992; *Topper & Brubaker*, 1985). Since timolol is able to block the stimulation of flow by intravenous epinephrine during sleep, it is thought that the circadian rhythm in aqueous flow is regulated by an endogenous hormonal agent that can be blocked when the agent is high, during waking hours, and is not present to be blocked during sleep (*Maus et al.*, 1996).

Carbonic anhydrase inhibitors

Systemic acetazolamide seems to reduce the flow rate during sleep by about the same percentage as it does during wakefulness (*McCannel. et al.*, 1992). Topical carbonic anhydrase inhibitors, dorzolamide and brinzolamide, also reduce flow rate but not as much as systemic carbonic anhydrase inhibitors (17% versus 30%) (*Maus et al.*, 1997). It is possible that topically applied drugs do not penetrate as well as systemically administered acetazolamide to sufficiently inhibit carbonic anhydrase in the ciliary body.

Adrenergic agonists

Clonidine and topical apraclonidine, alfa 2-adrenergic agonists, have been shown to suppress aqueous production by 20-30% (*Gharagozloo et al.*, 1988; *Lee et al.*, 1984; *Schadlu et al.*, 1998). Unlike timolol apraclonidine is capable to suppress flow during sleep (*Koskela and Brubaker*, 1991). Brimonidine increases uveoscleral outflow but does not appear to increase outflow facility in humans (*Toris et al.*, 1995).

Epinephrine is a direct adrenergic agonist of alfa1, alfa 2, and beta-receptors having a variable effect on aqueous flow. Results regarding whether it increases trabecular or uveoscleral outflow remain controversial (*Wang et al.*, 2002).

Table 4. First-line drugs. Table adapted from Terminology and Guidelines for Glaucoma – 4 Edition by EGS-European Glaucoma society, PubliCom.

CLASS	Compound	Mode of action	IOP reduction
Prostaglandin analogues	Latanoprost 0.005% Tafluprost 0.0015% Travoprost 0.003-0.004%	Increase in uveoscleral outflow	25-35%
Prostamide	Bimatoprost 0.01-0.03%		
Beta-receptor antagonists	Nonselective: Timolol 0.1-0.25-0.5% Levobunolol 0.25% Metilpranolol 0.1-0.3% Cartelol 0.5-1.0% Befunolol 0.5%	Decreases aqueous humour production	20-25%
	Beta-1-selective: Betaxolol 0.5%		±20%
Carbonic anhydrase inhibitors	Topical: Brinzolamide 1% Dorzolamide 2%	Decreases aqueous humour production	20%
	Systemic: Acetazolamide Methozolamide Dichlorphenamide		30-40%
Alpha-2 selective adrenergic agonists	Apraclonidine 0.5-1.0%	Decreases aqueous humour production	25-35%
	Brimonidine 0.2%	Decreases aqueous humour production and increases uveoscleral outflow	18-25%
	Clonidine 0.125-0.5%	Decreases aqueous humour production	

Table 5. Second-line drugs. Table adapted from Terminology and Guidelines for Glaucoma – 4 Edition by EGS-European Glaucoma society, PubliCom.

CLASS	Compound	Mode of action	IOP reduction
Non selective adrenergic agonists	Epinephrine 0.25-2.0% Dipivefrin 0.1%	Decreases aqueous humour production and may increases uveoscleral outflow	15-20%
Parasympathomimetics (cholinergic drugs)	Direct-acting: Pilocarpine 0.5-4% Carbachol 0.75-3%	Facilitates aqueous outflow by contraction of the ciliary muscle, tension on the scleral spur and traction on the trabecular meshwork	20-25%
	Indirect-acting: Demecarium bromide 0.125-0.25% Ecothiophate iodide 0.03% Diisopropyl fluorophosphates 0.025-0.1%		15-25%
Osmotics	Oral: Glycerol Isosorbide Alcohol	Dehydration and reduction in vitreous volume	15-20%
	Intravenous: Mannitol Urea	Posterior movement of the iris-lens plane with deepening of the AC	15-30%

5.2.2. Laser treatment: trabeculoplasty

Laser trabeculoplasty is a noninvasive technique employed to reduce the IOP in OHT, primary-open angle, exfoliative and pigmentary glaucoma when the IOP is not satisfactorily controlled with medications or as primary treatment if selective laser trabeculoplasty is used.

The most frequently used lasers are argon continuous-wave laser (**Argon laser trabeculoplasty)** and Q-switched, short pulsed and frequency-doubled neodymium: yttrium aluminium garnet (Nd:YAG) laser (**Selective laser trabeculoplasty**).

Argon laser trabeculoplasty (ALT) was introduced by *Wise* and *Witter* in 1979. (*Wise & Witter*, 1979) Its use gained popularity in the 1990s after the *Glaucoma Laser Trial* (1990) showed that ALT was at least as effective as initial treatment with timolol maleate 0.5%. However, the visible thermal damage, late pressure rise and treatment failure reduced its subsequent use.

Selective laser trabeculoplasty (SLT) was described by Latina and Park in 1995 (*Latina & Park.,* 1995) and approved by Food and Drug Administration in 2001. SLT uses a frequency-doubled, Q-switched, Nd: YAG laser and delivers a 400-μm-diameter treatment spot in 3 ns. The power ranges between 0.2 and 1.7 mJ with up to 120 applications per eye (**Table 6**; *see page 44*). Contrary to ALT, which causes visible thermal damage to the TM, SLT selectively targets the pigmented trabecular cells without damaging the adjacent non-pigmented meshwork structures.

SLT is based on a process called selective photothermolysis. This process involves targeting a specific cromophore with a thermal relaxation time (TRT) greater than the pulse duration of the laser emission so that laser energy is absorbed by the target cromophore confining heat diffusion. The TRT is the time required for the dissipation of thermal energy induced by the laser process. Melanin has a TRT of approximately 1 μs while the SLT pulse duration is 3ns, a pulse sufficiently short enough to eliminate the possibility of cooling of irradiated targets by the transfer of thermal energy to non-irradiated tissues (*Kagan et al.*, 2014).

Table 6. Selective laser trabeculoplasty versus argon laser trabeculoplasty

Laser characteristics	Selective laser trabeculoplasty	Argon laser trabeculoplasty
Spot size	400 µm	50 µm
Energy output	0.2-1.7 mJ	500-1000mW
Pulse duration	3 ns	0.1 s
Fluence	6 mJ/mm	40,000 mJ/mm

SLT is an effective treatment to reduce IOP in various types of OAG including POAG, normotensive glaucoma (NTG, *Liu and Birt*, 2012), steroid-induced glaucoma, pigmentary and pseudoexfoliative glaucoma (*Wong et al.*, 2015). SLT has also been used as a prophylaxis for anticipated steroid-induced glaucoma (*Bozkurt et al.*, 2011), after unsuccessful reduction of IOP after iridotomy (*Ho et al.*, 2009), deep sclerectomy (*Baykara et al.*, 2013), phaco-trabectome (*Töteberg-Harms & Rhee*, 2013) and after retinal detachment repair with silicone oil. (*Alkin et al.*, 2014) A recent meta-analysis reported the IOP reduction at ≥12 months ranges from 6.9% to 35.9% (*Wong et al.*, 2015). In comparison with ALT and medications, SLT was found to be non-inferior in terms of mean IOP reduction, reduction in numbers of medications and treatment success. Since most studies comparing SLT and medications were on patients with newly diagnosed glaucoma, the meta-analysis provided enough evidence that SLT can be used as primary treatment as well as adjunctive therapy when IOP is not controlled with maximally tolerated medication.

SLT is a low-risk procedure, with fewer complications than ALT. The most common complications are a transient elevation of IOP, iritis, eye pain or discomfort. (*Song et al.*, 2005; *Latina et al.*, 1998). The inflammatory response triggered by SLT may be responsible for anterior chamber reaction. This complication, including cells, flare and conjunctival injection has been commonly reported in several studies, with an incidence up to 83% (*Latina et al.*, 1998). The inflammation tends to settle down without medication but a short course of topical ant-inflammatories (steroidal or non-steroidal) is recommended.

An IOP increase of more than 5 mmHg should be considered significant and treated with additional topical or oral glaucoma medications. This spike of at least 5 mmHg can occur in ≤10% and ≥35% of patients with and without the use of topical alpha-2 adrenergics prophylaxis, respectively. It tends to resolve quickly with or without antihypertensive medications. However, patients with heavily pigmented angles may be at risk for high post-laser IOP spikes (*Bettis et al.*, 2016; *Harasymowycz et al.*, 2005). Other complications are headache and photophobia, corneal haze and abrasion, interstitial keratitis, hyphema, pigment dispersion, cystoid macular oedema and severe iritis with choroidal effusion. Peripheral anterior synaechia may also happen with SLT.

The success rate in achieving a 20% IOP reduction varies from 55% to 82% at 6-12 months when treating at least 180º. The lowest success rate was obtained by *Liu & Birt* (2012). In their study the baseline IOP was below 20 mmHg since patients with NTG were included. The success rate in this group was only of 22%. When less restrictive success definition is applied, the rate increases to 75-97% at 6-12 months follow-up (*De Keyser et al.*, 2016). The hypotensive effect of SLT decreases as with ALT with time. *Bovell et al.* (2011) reported success rates of 71% at 1 year, 52% at 2 years, 44% at 3 years, 38% at 4 years and 25% at 5 years. However, SLT may be repeated as early as 6 months after initially successful SLT fails with similar success rates (*Hong et al.*, 2009). Higher baseline IOP is the only factor that seems to predict SLT success. Age, race, glaucoma type and severity, trabecular meshwork pigmentation, pseudoexfoliation, number of medications, previous laser trabeculoplasty, iridotomy, hypertension, diabetes, phakia or pseudophakia did not influence on SLT response (*De Keyser et al.*, 2016).

The mechanism of action of SLT is not fully understood but is likely to reduce the IOP by increasing the trabecular outflow facility (G*oyal et al.*, 2010). It seems the effect is more biological and cellular than mechanical or thermal, with macrophagues from the spleen recruited into the TM via cytokines to remove debris from the TM and extracellular matrix turnover. Histological studies have demonstrated minimal coagulative or mechanical damage with only few cracks in the corneoscleral sheets and few endothelial cells with disrupted intracytoplasmic pigment granules and vacuoles.

(*Cvenkel et al.*, 2003; *Kramer & Noecker*, 2001) There is controversy whether SLT efficacy decreases with the use of concomitant prostaglandins as they may share similar mechanism of action and may compete with one another (*Ayala & Chen*, 2012; *Hirn et al.* 2012; *Kara et al.*, 2011; *Latina & Gulati*, 2004; *Singh et al.*, 2009). A retrospective study reported a lower success rate at 1 year following SLT in the previously prostaglandin analogues treated eyes (50%) compared to the timolol/dorzolamide fixed combination group (78.6%; *Kara et al.*, 2011). However, other studies demonstrated no difference in IOP reduction between eyes that had received prostaglandins and eyes that did not receive prostaglandins (*Ayala & Chen*, 2012; *Hirn et al.* 2012; *Singh et al.*, 2009). An in vitro study, using cultured human SC cells transfected with a plasmid construct containing the gene for the protein zonula occludens-1, demonstrated SC cells exposed to factors secreted by lasered cells and cells exposed to prostaglandin analogues had similar intercellular junction disassembly while increasing the permeability of the SC cells. Cells exposed to non-prostaglandin analogues (brimonidine, timolol, and dorzolamide) did not show intercellular junction disassembly (*Alvarado et al.*, 2009). It is also not clear whether the use of ant-inflammatory therapy influences the hypotensive effect of the laser but a recent randomised controlled study found the use of topical prednisolone, ketorolac or tears as placebo did not affect SLT response.

JUSTIFICATION

1. The effect of race on aqueous humor dynamics

Glaucoma is a treatable condition and is the second most common cause of blindness in Europe and worldwide. Primary open-angle glaucoma (POAG) is four to eight times more prevalent in black Africans and African-Caribbeans than in whites (*Friedman et al.* 2006; *Leske et el.* 1994; *Mason et al.* 1989; *Tielsch et al.* 1991). Several studies have demonstrated differences in structural and biometric parameters and risk factors for glaucoma development among ethnic groups. Black subjects have thinner corneas (*Rosa et al.,* 2001; *Shimmyo et al.,* 2003; *Brandt et al.,* 2001; *Kniestedt et al.* 2006) and larger optic discs (*Quigley et al.,* 1990; *Varma et al.* 1994; *Zangwill et al.,* 2004) and may have thinner retinal nerve fibre layers (*Poinoosawmy et al.,* 1997). The literature however, provides few insights into the potential racial differences in aqueous humor dynamics. Some studies on intraocular pressure (IOP) have reported higher IOP levels in blacks than in whites (*Coulehan et al.,* 1980; *David et al.,* 1978; *Friedman et al.* 2006), whereas others have found no differences (*AGIS,* 1998) or even lower IOPs in black subjects (*Broman et al.,* 2007). The therapy for glaucoma is preventive and the aim of therapy is for the disease to never impact on a patient's lifestyle. The cause of glaucoma is thought to be due to a direct damage to the trabecular meshwork (TM) reducing aqueous drainage through the trabecular pathway, but the relative damage to the outflow pathway or the aqueous production are probably partly determined by racial origins. Considering patients of African origin are at higher risk of losing vision than white Caucasians and the large black population Guy's and St Thomas' NHS foundation trust (GSTT) serves, we believe this is a particularly important field.

Since the aqueous dynamics in this population had not been yet studied, we proposed a cross-sectional observational study of different racial groups of patients with POAG to determine the relative contributions of altered aqueous production and drainage to the development of glaucoma in these patients.

2. The effect of selective laser trabeculoplasty on aqueous humor dynamics

Although the exact mechanism of action of selective laser trabeculoplasty (SLT) remains unclear, a previous study undertaken in the hospital showed that SLT reduces IOP by increasing trabecular outflow facility in patients with untreated POAG and ocular hypertension (OHT). We demonstrated a 29% reduction in IOP with only a 37.5% increase in outflow (Goyal et al., 2010). The increase in outflow facility, although could not explain fully the observed reduction in IOP, suggested that SLT could also affect other aqueous dynamics parameters, such as aqueous production, uveoscleral outflow, or episcleral venous pressure. Therefore the second part of the study was undertaken to elucidate whether SLT affects any other AHD parameter besides the outflow facility.

HYPOTHESIS

The working hypothesis were:

1) Development of glaucoma in people of African origin is caused by a greater increase in outflow resistance than in Caucasians without a compensatory reduction in aqueous production.

2) Selective laser trabeculoplasty may increase uveoscleral outflow as well as trabecular outflow facility.

OBJECTIVES

The aim of this Thesis was to study the aqueous humor dynamics (AHD) in patients with ocular hypertension (OHT) and primary open glaucoma (POAG) and analyse the influence of race/ethnicity and the effect of selective laser trabeculoplasty (SLT) on the AHD of such individuals. To do such work the following objectives were proposed:

Main Objectives

1) To compare the baseline AHD parameters in white Caucasian and patients of African origin with previously untreated POAG and OHT.

2) To investigate the effect of primary SLT on outflow facility and aqueous flow rate in a subset of these patients regardless their ethnicity.

Secondary Objectives

3) To study the influence of age on AHD parameters.

4) To determine de differences in AHD parameters between patients with POAG, OHT and healthy individuals.

5) To study the influence of IOP on aqueous production and outflow.

6) To study the differences in axial length (AXL), anterior chamber depth (ACD) and central corneal thickness (CCT) between white Caucasian and patients of African origin.

METHODOLOGY

1. Study design and recruitment

The research was carried out in the clinical Aqueous Dynamics Laboratory at St Thomas' Hospital in London (United Kingdom) established by Mr Kin-Sheng Lim. Consecutive new eligible patients referred to the glaucoma clinic were invited to participate. A patient information leaflet was provided at the initial contact, and signed consent was obtained before the baseline measurements and treatment.

Participants enrolled in this prospective, observational, controlled study were divided into four groups: (1) white subjects with primary open angle glaucoma (POAG) or ocular hypertension (OHT), (2) black subjects with POAG or OHT, and (3) white healthy ocular normotensive (ONT) volunteers and (4) black healthy ONT volunteers as control groups.

These patients were free to choose between prostaglandins or selective laser trabeculoplasty (SLT) as primary treatment. Patients that were treated with SLT were invited to have, after baseline aqueous dynamics measurements, a second set of aqueous dynamics measurements 3 months after the laser treatment.

Ethics approval for this study was obtained from the St Thomas's local research ethics committee. This research followed the tenets of the Declaration of Helsinki (Seoul, Republic of Korea, October 2008). A patient information leaflet was provided at the initial contact, and signed informed consent obtained before the baseline measurements and treatment.

2. Inclusion and Exclusion Criteria

Inclusion criteria were newly diagnosed, previously untreated adult POAG patients or patients with OHT that did or did not require treatment, both with intraocular pressure (IOP) > 21 mmHg at the screening visit. Glaucoma was diagnosed based on abnormal visual field testing and corresponding disc changes once seen by a fellowship-trained glaucoma specialist. Healthy volunteers had no ocular problems (other than refractive error) and IOP at screening <22 mmHg.

Exclusion criteria were secondary glaucomas including pigment dispersion syndrome and pseudoexfoliation, normotensive glaucoma, primary angle closure, history of uveitis, ocular trauma, intraocular or keratorefractive surgery, use of systemic medication that may affect aqueous humor production such as b-blockers, history of allergy or hypersensibility to fluorescein, and any abnormalities preventing reliable IOP or fluorophotometric readings.

Patients with unreliable fluorophotometric scans and/or tonography tracings in either one of the 2 sets of measurements were also excluded.

3. Data Collection and Outcome Measures

Data including age, sex, race, intraocular pressure (IOP), tonographic outflow facility, aqueous flow rate, central corneal thickness (CCT), axial length (AXL), anterior chamber depth (ACD), cup-to-disc ratio (CDR), mean deviation (MD) in visual field testing and amount of fluorescein drops and time of instillation were recorded.

The ***primary outcome measures*** were:

- IOP: differences between groups/change from baseline after SLT measured in mmHg by Goldmann applanation tonometer.
- Outflow facility: differences between groups/change from baseline after SLT measured in μL/min/mm Hg by Schiøtz tonometer.
- Aqueous flow rate: differences between groups/change from baseline after SLT measured in μL/min by fluorophotometry.
- Uveoscleral outflow: differences between groups measured in μL/min and calculated using the Goldmann equation.

The ***secondary outcome measures*** were:

- Age, measured in years.
- Central corneal thickness (CCT), measured in μm.
- Axial length (AXL) and anterior chamber depth (ACD), measured both in mm.

4. Measurements

At the screening visit, all patients underwent a clinical ophthalmological examination including visual acuity, slit-lamp examination, gonioscopy, ACD and AXL (IOL Master; Carl Zeiss Meditec Inc., Dublin, CA), CCT (Pachmate DGH 55; DGH Technology Inc., Exton, PA), IOP measured by Goldmann applanation tonometry (GAT), visual field tests (Humphrey automated white on white, 24-2 SITA standard), and dilated fundoscopy.

All patients had IOP, outflow facility, and aqueous flow rate measurements performed on the day of treatment and at least 3 months after the selective laser trabeculoplasty (SLT) treatment.

The night before (22:00 hours) the fluorophotometric scans, participants self-administered from 3 to 6 drops of fluorescein sodium 2% (Minims; Bausch & Lomb, Kingston-upon-Thames, UK) topically into both eyes at 5-minute intervals depending on their ages (age, ≤25 years, 5 to 6 drops; age 26–35 years, 4 drops; >35 years of age, 3 drops)(*Brubaker et al.*, 2001) Fluorophotometry was performed in both eyes with a scanning ocular fluorophotometer from 9:00 to 12:00 hours (FM-2, Fluorotron Master ocular fluorophotometer; OcuMetrics, Mountain View, CA). The *aqueous flow rate* was determined using dedicated software provided with the fluorophotometer. Duplicate or triplicate scans were collected and repeated at 1-hour intervals for four measurements to determine the aqueous flow rate (Ft). Following each set of scans, IOP was measured using pneumotonometry (**Figure 11-13**) Model 30 Classic; Reichert Ophthalmic Instruments, Depew, NY); *IOP* was recorded as the arithmetic mean of a total of 12 measurements per eye: 3 measurements every hour alternating between eyes. Patients with IOP >21 mmHg on the screening day may have had IOP of 21 mmHg or less thereafter. Before each measurement, the instruments were calibrated according to the manufacturer's instructions, the test procedure explained to the patient, and the importance of fixation stressed.

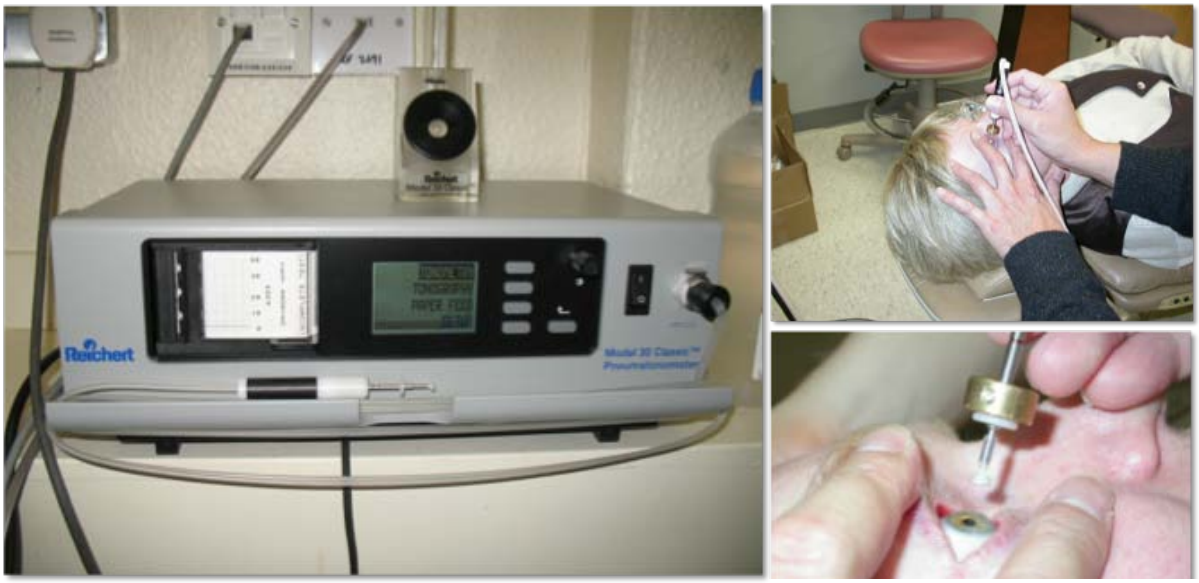


Figure 11-13: Pneumotonometer (**Model 30 Classic; Reichert Ophthalmic Instruments, Depew, NY**).

The Yablonski Protocol of estimating aqueous humor flow rate was used (*Schenker et al.*, 1981). Aqueous humor flow is the volume of aqueous humor produced by the ciliary body per unit of time.

$$Ft = Ko \cdot Va$$

where, *Ft* is the aqueous humor flow (µL/min), *Va* is the volume of the anterior chamber (µL), and *Ko* is the loss coefficient due to bulk flow and diffusion from the anterior chamber (minutes^{-1}). *Ko* can be thought of as the fraction of anterior chamber volume cleared of fluorescein every minute, due to aqueous flow **(Figure 14 & 15)**.

To calculate the aqueous flow, the program uses default variables that can be changed by the operator as necessary:

- Corneal volume default value, 70 µL
- Anterior chamber volume default value, 174 µL
- CCT of each patient, in µm. We introduced each patient's CCT instead of using the 500-µm default value to correct for the depth of the focal diamond. However, the corneal volume does not change if a different corneal central thickness is entered.

Once the relationship between cornea and anterior chamber concentrations of fluorescein becomes steady, the program can determine Ko and aqueous flow (Ft), as described in detail elsewhere. (Yablonski et al., 1978)

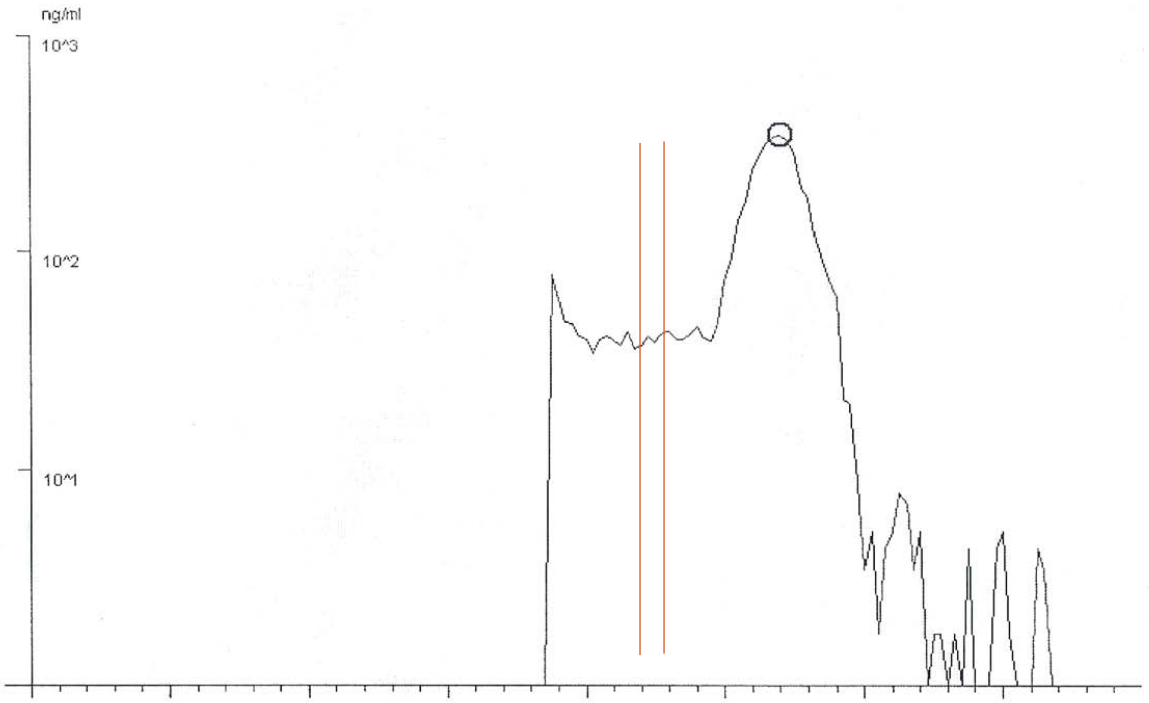
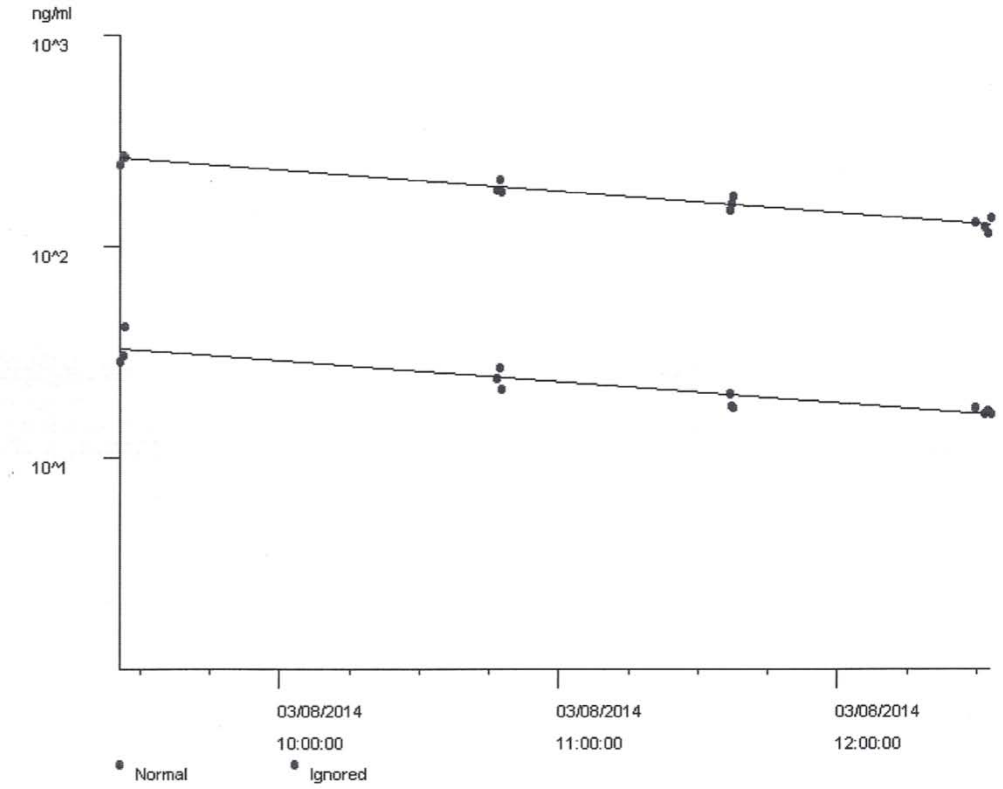


Figure 14: Graph obtained with each scan to measure the fluorescence of the cornea and anterior chamber at different time-points using the Fluorotron Master. The peak (circle) represents the corneal measurement and the flatter area, the concentration in the anterior chamber (between lines).



Protocol: Aqueous Turnover
Eye: Left Eye
Cornea Thickness (um): 527
r(ca): 1.6
Cornea Chamber Volume (uL): 70
Anterior Chamber Volume (uL): 174
Cornea Slope (min^-1): -0.0038961
Cornea Correlation Coefficient: 0.97653
Anterior Chamber Slope (min^-1): -0.0038225
Anterior Chamber Corellation Coefficient: 0.92717
D(average): 8.0108
Ka.ca (min^-1): 0.0031041
Kc.ca (min^-1): 0.0048225
Ko (min^-1): 0.016297
Aqueous Turnover (ul/min): 2.8357

Scan Time	Cornea	Anterior Chamber
03/08/2014 09:25:49	245.260167721979	28.6383027254289
03/08/2014 09:26:13	273.616616153289	30.7856865387183
03/08/2014 09:26:33	267.476534929069	42.022048401818
03/08/2014 10:46:47	185.616061097397	23.6557541632917
03/08/2014 10:47:26	208.28199874159	26.7097408947201
03/08/2014 10:47:49	181.435538853376	21.3136673309543
03/08/2014 11:36:55	148.169049388244	20.2347076063599
03/08/2014 11:37:20	159.543431905653	17.5399907275646
03/08/2014 11:37:40	172.102026460551	17.4137100743638
03/08/2014 12:29:46	129.836102270036	17.4318764153651
03/08/2014 12:31:50	123.90416265435	16.3240813877306
03/08/2014 12:32:17	116.012339251824	16.8411394645647

Figure 15: Fluorotron output sheet where the concentration decay of fluorescein in the cornea and anterior chamber throughout a 3-hour period used to calculate the aqueous turnover is shown. The graph shows this decay on a semi-log scale. The upper line is corneal fluorescein concentration, and the lower line is anterior chamber fluorescein concentration. The slope of the two lines and the distance between them indicate aqueous turnover and epithelial permeability. Special software is available for the Fluorotron for automatically calculating these values.

The *coefficient of aqueous outflow or outflow facility* (C) was measured with an electronic Schiøtz tonographer (Model 720; Berkeley Bioengineering, Inc., San Leandro, CA) at 10 AM **(Figures 16 & 17)**. The facility of outflow was measured from the rate of decay of IOP in the supine position during application of a recording Schiøtz tonometer over a period of 4 minutes with a standard 5.5-gr weight (*Grant*, 1951). When the IOP is over 30mmHg, the 7.5gr-weight should be employed. The R values of the curve at every 30-second time point were manually entered into the McLaren tonography computer program (*Lim et al.*, 2008). The program fits a second-degree polynomial by least-squares to the nine data points and determines by extrapolation the best-fit values for time 0 and time 4 minutes. Outflow facility was calculated using the 1955 scale approved by the *Committee on Standardization of Tonometers* (*Posner*, 1957).

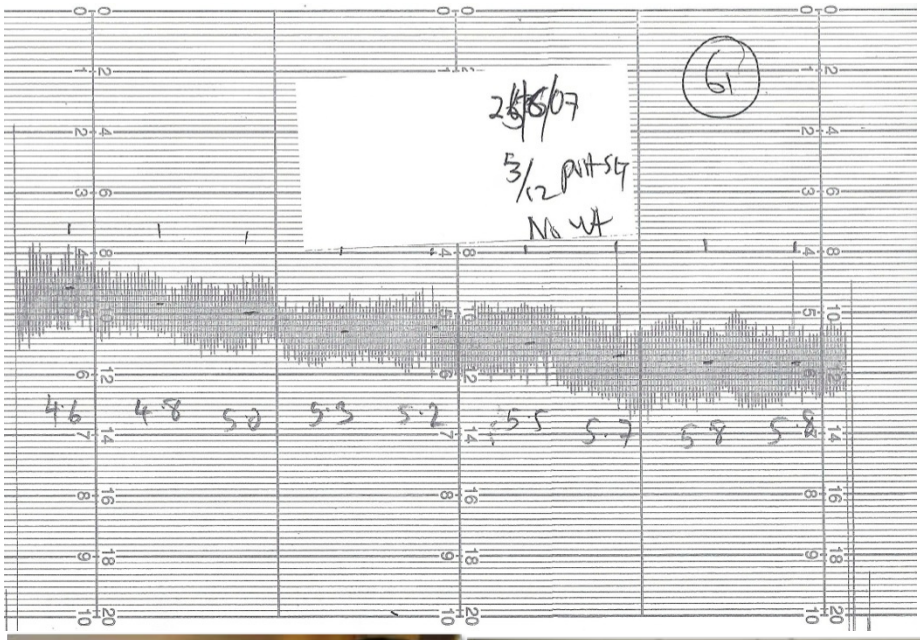


Figure 18: Schiøtz tonographer tracing 3 months after SLT treatment. Readings at every 30-second time point that best fit the line along the curve were taken to calculate outflow facility.

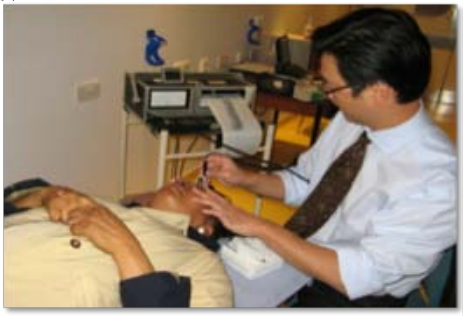
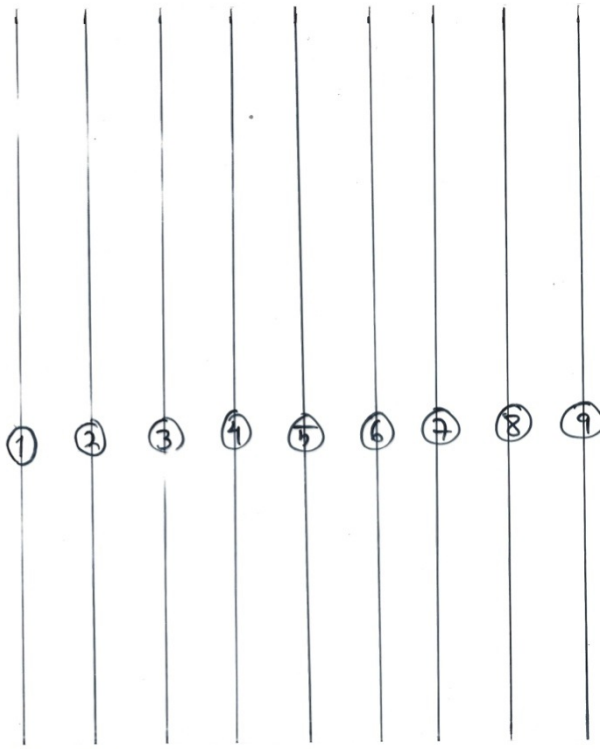
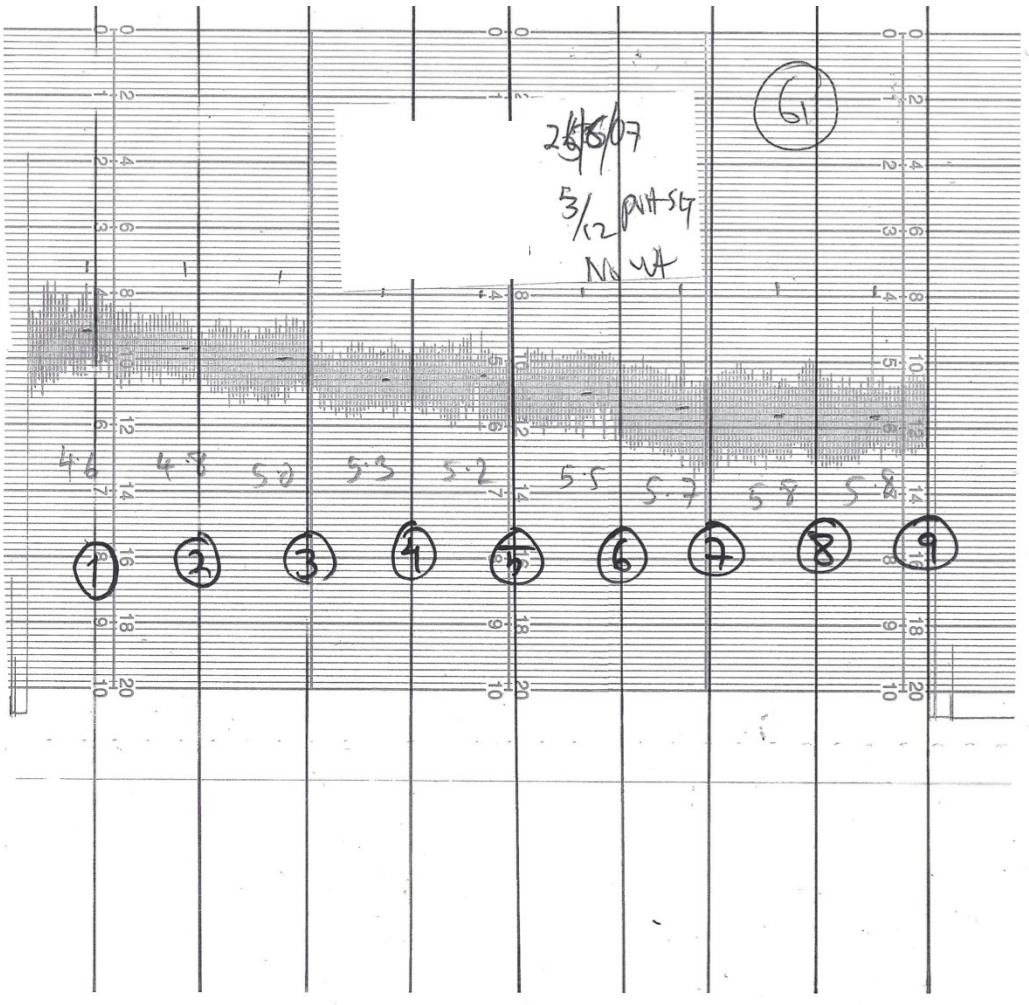
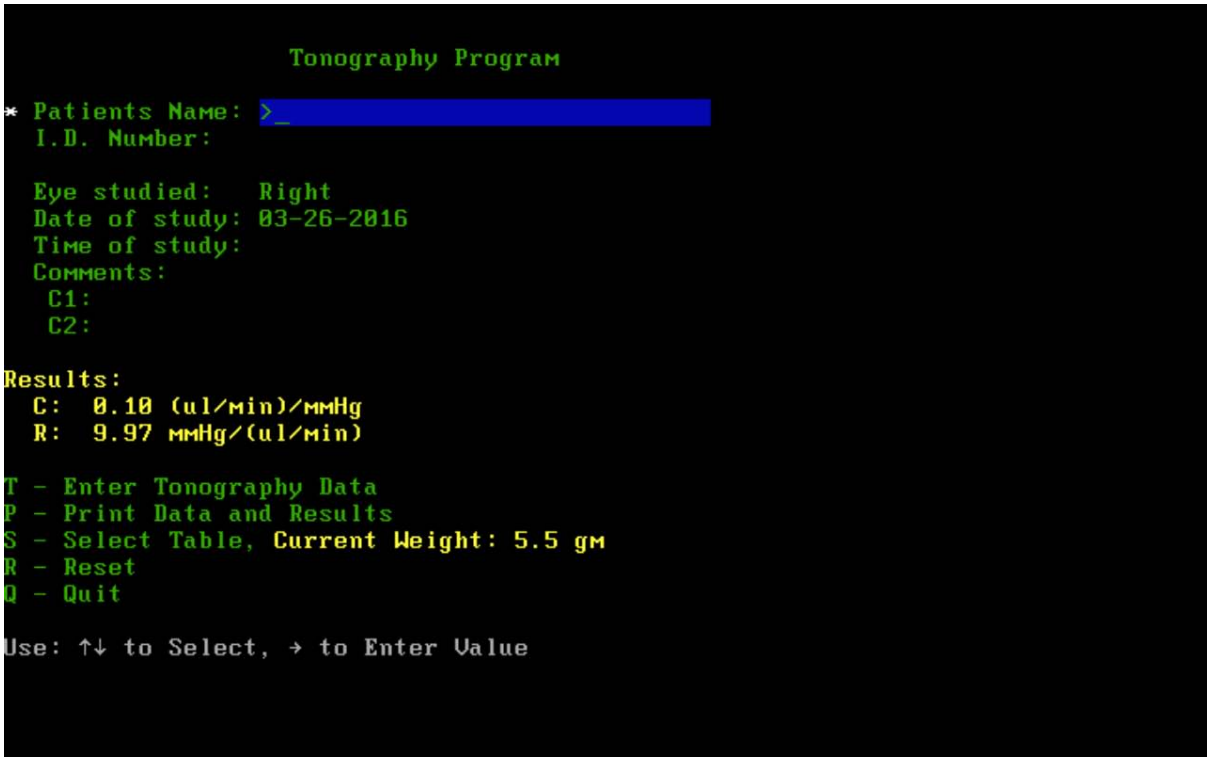


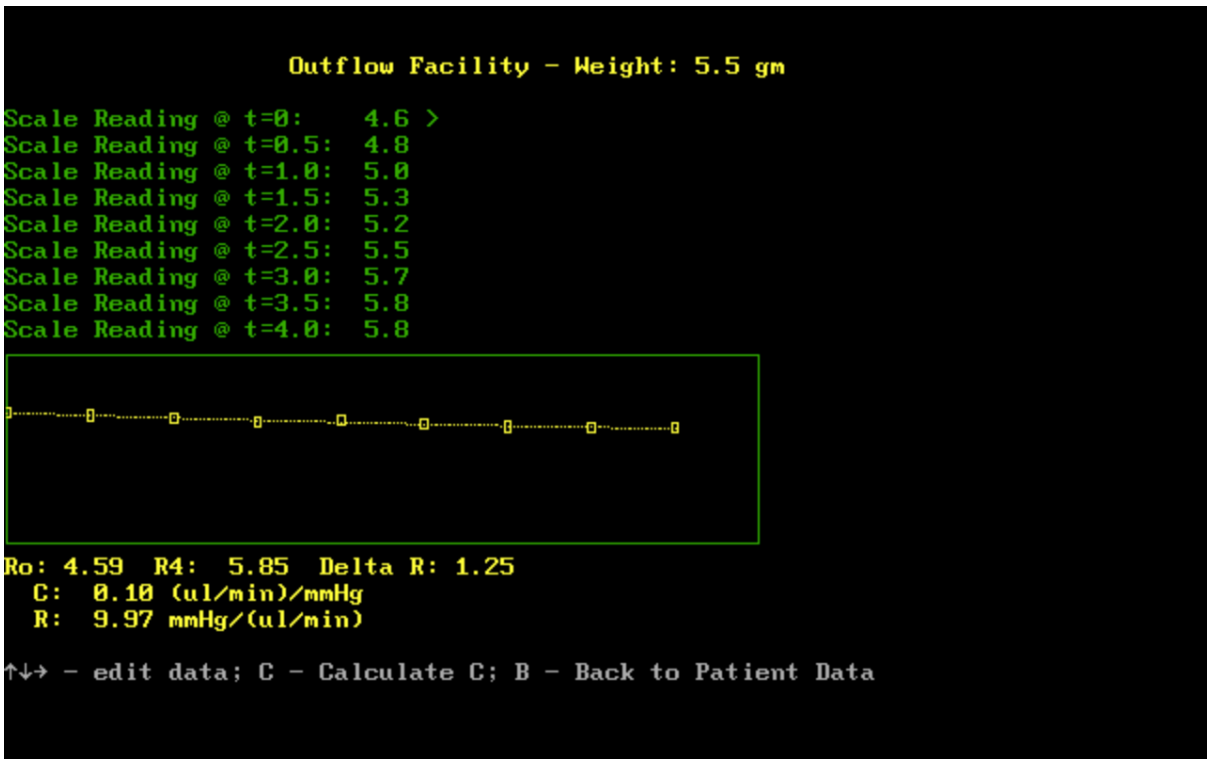
Figure 16 & 17: The Schiøtz tonographer (model 720; Berkeley Bioengineering, Inc., San Leandro, CA) used in this study (left). Mr KS Lim performing tonography to one of the participants (right).



Figures 21 & 22: A clear writing plastic sheet was used to identify the 9 time points (*R values*) of the curve that best fit the line along the curve (above). The plastic sheet was placed over each of the tracings and the points marked as shown (below). Note in the picture the vertical lines of the plastic sheet do not match the marks where the measurements were taken as it is only meant to be explanatory.







Figures 19 & 20: McLaren computer Tonography Program. The nine R values obtained from the curve were entered into the program to calculate the coefficient of aqueous or outflow facility(C). A standard weight of 5.5 g was used in most of the cases.

Uveoscleral outflow (Fu) was calculated using Goldmann's equation (*Brubaker*, 2004) with an assumed episcleral venous pressure of 8, 9, 10, or 11 mmHg (*Brubaker,* 2001b; *Lim. et al.,* 2008). Ft is the rate of aqueous humor formation, *C* is the coefficient of aqueous outflow or tonographic outflow,facility IOP is the intraocular pressure before placing the tonometer on the cornea, *Pv* is the episcleral venous pressure, and *Fu* is the uveoscleral outflow.

$$F_t = C \times (IOP - P_V) + F_u$$

$$F_u = F_t - C \times (IOP - P_V)$$

Only one randomly (Excel random number generator; Microsoft, Redmond, WA) chosen eye per participant was included in the data analysis, when both eyes fulfilled the inclusion criteria.

If additional glaucoma medical treatment was started to achieve the target pressure within 3 months after the SLT treatment, patients were excluded from the study and the second set of measurements was not performed.

5. Selective laser trabeculoplasty treatment

All patients underwent 360-degree selective laser trabeculoplasty (SLT) treatments that were performed by three different physicians. Pilocarpine 2% to 4% drops (Minims, Pilocarpine nitrate; Chauvin Pharmaceuticals Ltd, Surrey, UK) were instilled half an hour before SLT. SLT treatment was performed using the Ellex Solo machine (Ellex, Adelaide, Australia), spot size of 400 mm, duration 3 nano-seconds. Starting energy level was 0.6 mJ. The energy level was titrated at the 3-o'clock position up to the point where champagne bubbles or minimal blanching was visible. A total of 90 to 100 laser burns were given using the Magna view gonioscopy lens (Ocular Instruments, USA) to visualise the trabecular meshwork. Apraclonidine hydrochloride 1% drops (Iopidine 1%; Laboratòires Alcon S.A., Kayserberg, France) were instilled before and after laser therapy to prevent the IOP spike after laser therapy. All patients had a standardised postoperative regimen of dexamethasone 0.1% eye drops (Maxidex;

Alcon Laboratories, UK) 4 times a day for 5 days to treat intraocular inflammation after SLT.

6. Data Analysis

Histograms and a Shapiro-Wilk test were performed to test for normality of distribution of data. A Shapiro-Wilk $W > 0.05$ was considered of normal distribution. Student's *t*-test and one-way analysis of variance (ANOVA) were used to compare continuous variables among groups. When data did not follow normality, nonparametric methods of analysis (Mann-Whitney U and Kruskal-Wallis tests) were used. Post hoc comparisons among groups were made when appropriate, by Holm's sequential Bonferroni method (HBonf). The 95% confidence intervals (CI) for the mean and median difference between pairs for each outcome measure were calculated. The median difference 95% CIs was estimated using Hodges-Lehman methodology. Linear regression analyses were used to determine the correlation of one parameter versus another parameter of aqueous humor dynamics and the correlation of age versus each parameter. Fisher's exact test was used to compare the number of patients with POAG and OHT in each racial group. Paired Student t tests were used to compare aqueous dynamics parameters before and after SLT treatment. A $P < 0.05$ was considered statistically significant. All analyses were performed using the statistical software SPSS 16.0 (SPSS, Chicago, IL).

RESULTS

1. Patient description

One hundred and one patients with OHT/POAG and 32 healthy volunteers were recruited into the study. Only 66 affected subjects and 25 controls were included in the data analysis. Poor-quality Schiøtz tonography tracings (n = 14), with excess noise or extreme excursions, or poor fluorophotometric scans with extremely high or low baseline corneal fluorescein concentration (n = 27), were identified in 41 participants and were therefore excluded from the analysis. Another participant was excluded for not having slept the night before the measurements were taken (*Reiss et al.*, 1984).

In the black POAG/OHT group, 13 (38.2%) subjects had OHT and 21 (61.8%) had POAG, compared with 18 (56.2%) subjects with OHT and 14 (43.8%) subjects with POAG in the white affected group ($P = 0.21$).

Baseline characteristics from each group and comparison of each AHD parameter between groups are shown in **Table 7** *(see page 76)*.

2. Aqueous humor dynamics comparison between black and white subjects and its relationship with intraocular pressure

2.1. Outflow facility

Both the black and white groups with POAG/OHT had significantly lower outflow facility than their matched control group (black, $P_{HBonf} < 0.001$; 95% CI, -0.11 to -0.02; white, $P_{HBonf} < 0.001$; 95% CI, -0,17 to -0.03), but no differences were found between the affected groups ($P_{HBonf} = 0.51$; 95% CI, -0.03 to 0.04). A weakly significant inverse relationship was observed between outflow facility and IOP, with a reduction of 0.008 ± 0.002 µL/min/mm Hg ($R^2 = 0.16$; $P < 0.001$; **Figure 23**; *see page 76*). This relationship remained after adjustment for age ($R^2 = 0.21$; $P < 0.001$). Facility of outflow appeared not to be linearly correlated to the stage of glaucoma damage measured by mean deviation (MD) in visual field testing ($R^2 = 0.002$; $P = 0.72$) and cup-to-disc ratio ($R^2 = 0.007$; $P = 0.51$).

Table 7. Comparison of baseline aqueous dynamics parameters among black and white patients with POAG or OHT and ocular normotensive volunteers(ONT).

Parameters	POAG/OHT						ONT						
	Black (n=34)			White (n=32)			Black (n=12)			White (n=10)			
	Median (mean)	IQR (SD)	Range (min, max)	Median (mean)	IQR (SD)	Range (min, max)	Median (mean)	IQR (SD)	Range (min, max)	Median (mean)	IQR (SD)	Range (min, max)	*P**
IOP, mmHg		4.44 (4.04)	20.96 (18.50, 39.46)	25.00 (24.66)	3.89 (3.05)	11.33 (19.92, 31.25)	18.79 (17.88)	4.25 (2.60)	7.88 (13.79, 21.67)	17.12 (16.52)	3.96 (2.90)	9.25 (11.25, 20.50)	***<0.001***†
Ft, µL/min	2.36 (2.43)	0.83 (0.58)	3.01 (1.37, 4.37)	2.40 (2.45)	0.88 (0.68)	2.74 (1.02, 3.77)	2.21 (2.09)	0.91 (0.55)	1.77 (1.24, 3.01)	2.34 (2.26)	0.42 (0.44)	1.60 (1.18, 2.78)	*0.23*
C, µL/min/mmHg	0.12 (0.13)	0.08 (0.07)	0.36 (0.03, 0.39)	0.11 (0.13)	0.13 (0.09)	0.41 (0.03, 0.44)	0.21 (0.21)	0.08 (0.08)	0.33 (0.07, 0.40)	0.27 (0.25)	0.22 (0.11)	0.33 (0.08, 0.41)	***0.002***†
Fu_8	0.43 (0.39)	1.62 (1.33)	6.58 (-4.01, 2.56)	0.66 (0.24)	2.78 (1.77)	7.42 (-4.54, 2.87)	0.18 (-0.03)	1.31 (0.96)	3.16 (-2.03, 1.12)	0.28 (0.05)	2.19 (1.25)	3.87 (-2.33, 1.54)	*0.83*
Fu_9	0.58 (0.53)	1.53 (1.27)	6.33 (-3.73, 2.59)	0.76 (0.37)	2.58 (1.68)	7.04 (-4.10, 2.93)	0.36 (0.18)	1.37 (0.90)	2.97 (-1.63, 1.33)	0.52 (0.30)	2.04 (1.17)	3.59 (-1.92, 1.67)	*0.90*
Fu_{10}	0.73 (0.66)	1.44 (1.21)	6.14 (-3.45, 2.69)	0.88 (0.51)	2.38 (1.60)	6.66 (-3.66, 2.99)	0.51 (0.39)	1.43 (0.85)	2.78 (-1.23, 1.54)	0.76 (0.55)	1.88 (1.09)	3.39 (-1.51, 1.88)	*0.94*
Fu_{11}	0.85 (0.79)	1.32 (1.16)	5.99 (-3.17, 2.82)	0.96 (0.65)	2.18 (1.52)	6.28 (-3.22, 3.05)	0.66 (0.60)	1.49 (0.80)	2.59 (-0.83, 1.75)	0.97 (0.81)	1.69 (1.02)	3.32 (-1.10, 2.22)	*0.95*

POAG: primary open angle glaucoma; OHT: ocular hypertension; ONT: ocular normotension; n: number of eyes; IQR: interquartile range; SD: standard deviation; IOP; intraocular pressure; Ft: aqueous flow rate; C: trabecular outflow facility; $Fu_{8\text{-}11}$: uveoscleral outflow with an assumed episcleral venous pressure of 8-11 mmHg; P: probability value, (*) calculated using one-way ANOVA or (†) Kruskal Wallis tests.

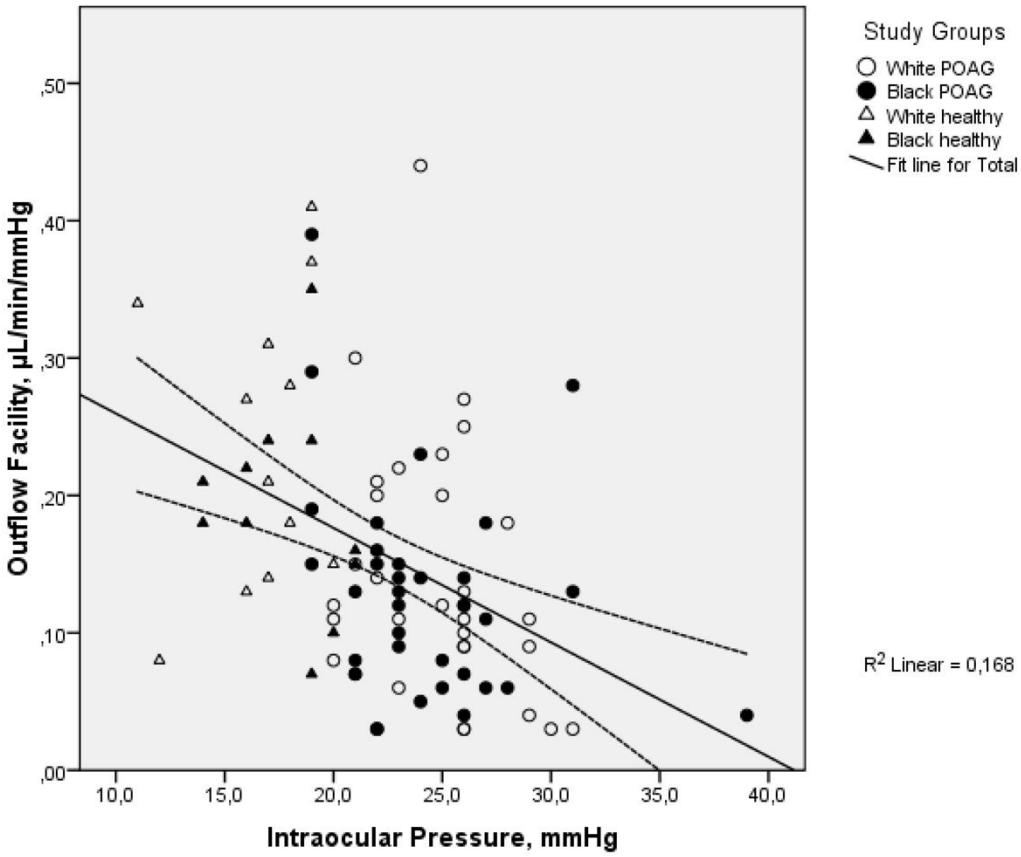


Figure 23: Scatter graph and bivariate regression analysis between the intraocular pressure (IOP) and tonographic outflow facility (C). The regression line is C = -0.008 x IOP + 0.343, P<0.001, and R^2 = 0.16 (95% confidence bands are shown).

2.2. Intraocular pressure

Subjects with POAG/OHT exhibited significantly higher IOP than their control counterparts ($P < 0.001$), but the black and white affected groups had comparable IOP ($P = 0.51$; 95% CI, -1.0 to 2.0).

2.3. Aqueous flow rate

The mean aqueous flow rates for each group ranged from 1.81 to 2.36 µL/min. The black control subjects had significantly lower aqueous production than did the black affected subjects ($P_{HBonf} = 0.01$; 95% CI, 0.17–0.90). The aqueous production did not differ significantly between the black and white affected groups ($P_{HBonf} = 0.95$; 95% CI, -0.29 to 0.30). Aqueous flow was independent of IOP ($R^2 = 0.03$; $P = 0.07$).

2.4. Uveoscleral outflow

The uveoscleral outflow was not different among groups, regardless of the assumed episcleral venous pressure. The 95% CI for the difference between the affected group medians for Fu10 (Pv = 10 mmHg) was -0.74 to 0.58 µL/min. Affected patients, independent of their racial origin (n = 66), had a mean Fu10 of 0.75 ± 1.36 µL/min (median, 0.50; range, -4.04 to 2.68) compared with 0.47 ± 0.95 µL/min (median, 0.52; range, -1.51 to 2.19; P = 0.51) in all control subjects (n = 25). Contrary to outflow facility, the relationship between uveoscleral outflow (Fu10) and IOP (R^2 = 0.007; P = 0.44) did not reach significance. Uveoscleral outflow and stage of glaucomatous damage were not related. Similar results were obtained with assumed episcleral venous pressures of 8, 9, and 11 mmHg.

Despite the pre-study sample size calculation based on the results of another study,19 post hoc analysis of our data showed that our study would be able to detect a difference of 10.2% in IOP, 9.7% in aqueous flow, and only 38% in outflow facility, with a power of 80% and α (two-sided) = 0.05, if those differences existed.

3. Age

The mean age of the study population was 58.7 ± 13.0 years. Age did not correlate with the outcome measures in any of the affected groups or in all participants combined (IOP, P = 0.58; C, P = 0.08; Ft, P = 0.56; and Fu10, P = 0.06).

4. Ocular Hypertension versus Primary Open Angle Glaucoma

Table 8 *(see page 79)* summarizes the aqueous dynamics parameters of the POAG and OHT subjects, which showed non-significant differences.

5. Axial length, anterior chamber depth & central corneal thickness

Black POAG/OHT subjects had significant thinner corneas than did the white POAG/OHT group (P_{HBonf} = 0.009; 95% CI, 8–43) but no other difference regarding CCT was observed between the rest of the groups. The mean CCT of all black participants was 540 ± 37 µm compared with 564 ± 36 µm in all white participants together (P = 0.002;

95% CI, 8 – 40). CCT did not correlate significantly with any of the aqueous dynamics parameters. AXL, ACD and CCT values are shown in **Table 9** *(see page 80)*.

Table 8. Comparison of baseline aqueous dynamics parameters between patients with POAG and OHT.

	POAG vs OHT						
	POAG (n=35)			OHT (n=31)			
Parameters	**Median (mean)**	**IQR (SD)**	**Range (min, max)**	**Median (mean)**	**IQR (SD)**	**Range (min, max)**	***P****
Age, years	62.00 (62.17)	20 (12.88)	55 (33, 88)	54.00 (56.42)	22 (13.03)	53 (30, 83)	*0.07*†
IOP, mmHg	23.25 (23.79)	4.04 (2.83)	12.75 (18.50, 31.25)	25.71 (25.24)	5.50 (4.18)	21 (18.50,39.46)	*0.13*
Ft, µL/min	2.44 (2.47)	0.89 (0.67)	3.35 (1.03, 4.38)	2.32 (2.40)	0.86 (0.59)	2.49 (1.20, 3.69)	*0.63*†
C, µL/min/ mmHg	0.12 (0.13)	0.07 (0.08)	0.41 (0.03, 0.44)	0.11 (0.13)	0.14 (0.09)	0.36 (0.03, 0.39)	*0.98*
Fu 8, µL/min	0.59 (0.45)	1.36 (1.45)	7.42 (-4.54, 2.87)	0.56 (0.17)	2.77 (1.66)	6.79 (-4.01, 2.77)	*0.54*
Fu 9, µL/min	0.77 (0.58)	1.29 (1.38)	7.04 (-4.10, 2.93)	0.67 (0.31)	2.51 (1.59)	6.55 (-3.73, 2.81)	*0.53*
Fu 10, µL/min	0.95 (0.71)	1.23 (1.32)	6.66 (-3.66, 2.99)	0.78 (0.45)	2.30 (1.51)	6.31 (-3.45, 2.85)	*0.51*
Fu 11, µL/min	1.01 (0.84)	1.17 (1.25)	6.28 (-3.22, 3.05)	0.89 (0.58)	2.13 (1.44)	6.07 (-3.17, 2.89)	*0.48*
CCT, µm	549.50 (547.67)	51.25 (39.60)	196 (438, 634)	554.50 (556.66)	50.75 (34.39)	129 (492, 621)	*0.33*†
MD, dB	-3.99 (-5.62)	5.22 (5.70)	30.52 (-29.97, 0.55)	-1.30 (-1.46)	2.23 (2.56)	15.53 (-7.58, 7.95)	***<0.001***

POAG: primary open angle glaucoma; OHT: ocular hypertension; n: number of eyes; IQR: interquartile range; SD: standard deviation; IOP; intraocular pressure; Ft: aqueous flow rate; C: trabecular outflow facility; $Fu_{8\text{-}11}$: uveoscleral outflow with an assumed episcleral venous pressure of 8-11 mmHg; CCT: central corneal thickness; MD: mean deviation in dB; P: probability value, (*) calculated using Mann-Whitney U or (†) Student t tests.

Table 9. Descriptive parameters of black and white patients with POAG or OHT and ocular normotensive volunteers (ONT).

Parameters	POAG/OHT						ONT						
	Black (n=34)			White (n=32)			Black (n=12)			White (n=10)			
	Median (mean)	IQR (SD)	Range (min, max)	Median (mean)	IQR (SD)	Range (min, max)	Median (mean)	IQR (SD)	Range (min, max)	Median (mean)	IQR (SD)	Range (min, max)	*P*
Age, years	59.50 (57.71)	24 (12.97)	49 (30, 79)	60.00 (59.75)	22.50 (12.44)	43 (40, 83)	49.50 (50.75)	8.00 (10.52)	36 (38, 74)	63.50 (59.00)	33.25 (17.14)	46 (33, 79)	*0.12**
ACD, mm	3.14 (3.19)	0.59 (0.38)	1.40 (2.59, 3.99)	3.23 (3.30)	0.46 (0.30)	0.99 (2.89, 3.88)	3.21 (3.22)	0.78 (0.37)	1.02 (2.78, 3.80)	3.28 (3.24)	0.35 (0.34)	1.16 (2.55, 3.71)	*0.65*†
AXL, mm	23.52 (23.67)	0.99 (0.83)	3.72 (22.12, 25.84)	23.56 (24.00)	1.73 (1.33)	4.72 (22.48, 27.20)	23.27 (23.45)	1.37 (0.77)	2.29 (22.34, 24.63)	23.70 (23.90)	2.00 (1.18)	3.46 (22.29, 25.75)	*0.89*†
CCT, µm	548 (539)	55 (37)	168 (438, 606)	569 (566)	44 (31)	121 (513, 634)	538 (535.98)	40.00 (29.19)	106 (479, 585)	558.50 (553.87)	103.74 (50.26)	131 (483, 614)	***0.03***†

POAG: primary open angle glaucoma; OHT: ocular hypertension; n: number of eyes; ONT: ocular normotension; IQR: interquartile range; SD: standard deviation; ACD: anterior chamber depth; AXL: axial length; CCT: central corneal thickness; P: probability value, (*) calculated using one-way ANOVA or (†) Kruskal Wallis tests.

6. Selective Laser Trabeculoplasty

Thirty consecutive patients with OHT/POAG treated with 360-degree SLT had baseline and post treatment aqueous dynamics measurements performed. Poor-quality Schiøtz tonography tracings with excess noise or extreme excursions (n = 6) and poor fluorophotometric scans with extremely high or low baseline corneal fluorescein concentration (n = 5) were identified in 11 eyes and were therefore excluded from the analysis. One patient was advised to use antihypertensive drops after SLT and was therefore excluded from the study. Eighteen eyes (9 OHT and 9 POAG) with a median follow-up time post SLT of 3.6 (2.8 to 11.0) months were included in the final analysis. The mean age of the study population was 56.7 ± 12.4 (30.8 to 79.3) years. Fourteen patients were male. No significant sex differences in pre laser and post laser aqueous dynamics parameters were found. The SLT treatment was well tolerated and no post-laser complications were observed. The mean total energy used was 95.5 ± 17.6 mJ (62 to 128 mJ; n = 14).

All study parameters were normally distributed. Differences between aqueous dynamics parameters at baseline and after SLT treatment are shown in **Table 10** *(see page 82)*. A statistically significant reduction in IOP of 5.0 ± 3.4 mmHg (21%) with a significant increase in trabecular outflow facility of 0.05 ± 0.06 mL/min/mmHg (55.5%) after SLT was observed. The change in trabecular outflow facility after SLT (-0.05 to 0.24 mL/min/mmHg) and the change in IOP from baseline were not significantly correlated (Spearman $\rho = 0.25$, $P = 0.30$). No significant change in aqueous flow rate was observed.

Table 10. Comparison of aqueous dynamics parameters before and after 360º SLT treatment

Parameters	Pre SLT treatment (n=18)			Post SLT treatment (n=18)			
	Mean	**SD**	**Range (min, max)**	**Mean**	**SD**	**Range (min, max)**	***P***
IOP, mmHg	24.0	3.0	12.2 (17.0, 29.2)	18.9	2.7	10.2 (15.2, 25.5)	*<0.001**
Ft, µL/min	2.24	0.83	2.69 (0.99, 3.69)	2.09	0.70	2.27 (1.10, 3.37)	*0.46*
C, µL/min/mmHg	0.09	0.05	0.18 (0.02, 0.20)	0.14	0.08	0.27 (0.03, 0.30)	*0.003**

n: number of eyes; SD: standard deviation; IOP; intraocular pressure; Ft: aqueous flow rate; C: trabecular outflow facility; P: probability value calculated with paired t Student test; (*): statistically significant with a P<0.05.

DISCUSSION

Previous aqueous dynamics studies have included black participants (*Toris et al.,* 2002) but this is the first study that specifically compared the aqueous dynamics between Africans/African Caribbeans and white Caucasians. Despite the anatomic differences reported between black and white populations, this study found no significant differences in the aqueous dynamics among patients with POAG/OHT of both racial groups.

We also investigated the effect of SLT on aqueous dynamics in treatment of naive subjects with newly diagnosed POAG and OHT. We found a significant reduction in IOP with an associated increase in tonographic outflow facility 3.6 months after SLT with no change in aqueous production. To our knowledge, this is the first study that investigates the effects of SLT on outflow facility and aqueous production, helping us to improve our understanding of its mechanism of action.

To measure the trabecular outflow facility, we used the classic electronic Schiøtz tonometer because compared to pneumotonography or fluorophotometry techniques, the Schiøtz tonometer gives an accurate assessment of outflow facility with less variability over time (*Lim et al.*, 2008). Rejection rate of 14 of 101 subjects due to poor-quality tonography is only slightly higher than that found in a previous study by the same researchers (*Lim et al.*, 2008).

Since *Grant* (1951) and *Becker* (1961) reported a reduction in outflow facility in patients with glaucoma many years ago, other groups have come to the same conclusion (Carol et al., 2002; *Martin et al.,* 1992). We found a similar reduction in the outflow facility in both affected racial groups without a significant increase in the aqueous production. The measurements performed with the Schiøtz tonometer are affected by ocular rigidity (*Gloster,* 1965). Indentation tonography makes no compensation for individual variations in ocular rigidity. The stiffer the eye, the greater the force needed to indent the cornea and displace the aqueous. Corneal hysteresis (CH) and corneal resistance factor (CRF) are dynamic measurements of the viscoelastic properties of the cornea that have been found to be lower in subjects of black African descent than in whites (*Broman et al.*, 2007; *Leite et al.*, 2010). Several studies have shown an increase in CH and CRF with increasing CCT (B*roman et al.*, 2007; *Shah et al.*, 2006; 2008).

The higher the CH, the longer the cornea takes to return to its original shape, implying greater stiffness. Therefore, higher outflow facility measurements could have been obtained in the black participants on the basis of decreased ocular rigidity rather than the true equality found among the groups. On the other hand, *Pallikaris et al.* (2005) measured in vivo the rigidity coefficient in 79 living human eyes and found no statistically significant correlation between ocular-rigidity coefficient and CCT. They reported that despite the low power to detect that correlation, CCT may have an effect on the corneal biomechanics, but may have less impact in ocular rigidity which involves the whole eye and not only the cornea.

Although it has been found to be reduced in beagle dogs with advanced glaucoma (*Barrie et al.,* 1985) and elevated in monkeys with laser-induced glaucoma (*Toris et al.*, 2000), the uveoscleral outflow was not significantly different between the white and black groups or the affected subjects and healthy volunteers. In humans with POAG, *Toris et al.* (2002), reported that uveoscleral outflow could be reduced in early stages of glaucoma, together with reduced trabecular outflow, but increases as the disease progresses to prevent the IOP from increasing further, but we did not find any differences between patients with OHT and POAG compared with normal subjects.

Overall, we found a 21% reduction in IOP that is comparable with previous SLT and ALT studies (*Brubaker & Liesegang,* 1983; *Goyal et al.*, 2010; *Nagar et al.*, 2005; *Thomas et al.,* 1982). The IOP-lowering effect of SLT was accompanied by a 55.5% increase in tonographic outflow facility. Although the increase in facility of outflow is greater than we previously reported, it is similar to other studies on the effect of ALT on aqueous dynamics (*Brubaker & Liesegang,* 1983; *Bergea˚ & Svedbergh,* 1992; *Thomas et al.,* 1982). However, as highlighted by *Goyal et al.* (2010), those studies were mainly done in medically treated patients, making any direct comparison difficult.

Bergea˚& Svedbergh (1992) reported a 33% reduction in IOP with a tonographic outflow facility increase of 63.5% in medically untreated eyes (n = 11) with POAG. However, their patients had higher baseline IOP and had only 180-degree ALT. *Brubaker & Liesegang's* (1983) study found similar IOP reduction (29%) and increase in tonographic outflow facility (64%) after ALT in medically treated patients (n = 17) using the same tonographic technique. They did not find any effect of ALT on aqueous

production. Another study by *Thomas et al.* (1982), obtained the same reduction in IOP (29%) and increase in outflow facility (64%) as *Brubaker & Liesegang* (1983) but in patients with different types of glaucoma who were on maximal-tolerated medical treatment. Despite the patients in those studies being already on medication before the laser treatment, they had similar baseline IOP as our previously untreated patients. Topical medications do not seem to affect SLT success. However, SLT efficacy has been positively associated with baseline IOP (*Ayala & Chen,* 2011; *Martow et al.*, 2011). Thus, regardless of whether patients are medically treated before the laser treatment, equivalent results would be obtained if patients had similar IOP before the laser therapy.

Yablonski et al. (1985) studied the aqueous dynamics changes after ALT (n=15) and found a 34% increase in tonographic outflow facility with no effect on the rate of aqueous humor production. They also observed a reduction in uveoscleral outflow that was attributed to the reduction in IOP after laser therapy.

We found that the rate of aqueous humor production was not affected by SLT. Our results suggest that the eventual mechanism of action of SLT is likely to be very similar to ALT. Both types of trabeculoplasty would reduce the IOP by increasing the outflow through the trabecular meshwork. *Van Buskirk et al.* (1984) suggested ALT might have a mechanical as well as a biochemical effect on the trabecular meshwork. *Kramer & Noecker* (2001) compared the histopathologic changes in the trabecular meshwork after ALT and SLT in human eye bank eyes. They demonstrated that both types of laser altered the ultrastructure of the trabecular meshwork endothelial cells. However, ALT was also associated with coagulative damage to the trabecular beams. They suggested that SLT might only have a selective biological effect on the trabecular meshwork while ALT would have an additional mechanical effect, responsible for the fibrosis and scarring. If ALT and SLT have similar mechanisms of action, the structural damage produced by ALT may be an unnecessary consequence of the argon laser, not contributing to the trabeculoplasty IOP-lowering effect (*Stein & Challa*, 2007). It is possible that immediately after the SLT, there may be some effect on the uveoscleral outflow secondary to the inflammatory response created by the laser treatment, which would subside after a few months. Unfortunately, because there are currently no

reliable clinical methods of measuring uveoscleral outflow (Fu), this theory remains highly speculative at best.

Pilocarpine and apraclonidine drops instilled before SLT treatment would not have interfered with our measurements, as their effect on outflow facility and aqueous flow will at maximum last for a few days. Apraclonidine has been associated with an increase in fluorophotometric outflow facility (*Toris et al.,* 1995), but there is no evidence that a-agonists affect tonographic outflow facility using the Schiøtz technique as was used in this study (*Toris et al.*, 1999a).

Larsson et al. (1995) and *Toris et al.* (2002) showed that the rate of aqueous production was not different in patients with glaucoma or ocular hypertension compared with healthy age-matched controls. This study confirmed this finding, and we also found no racial difference in the aqueous production rates.

Despite the number of patients with POAG being higher in the black affected group than in the white affected group, this disproportion between both racial groups would not have interfered in our analysis, since OHT and POAG patients have comparable aqueous dynamics. Contrary to some studies (*Coulehan et al.,* 1980; *David et al.,* 1978; *Friedman et al.* 2006) we found black and white participants with POAG/OHT had similar IOP, but this is likely to be due to the bias in our sampling as patients with IOP higher than 35 mmHg at the screening visit, according to our ethics committee recommendations, would have been excluded from the study, to avoid delaying the instauration of the medical treatment. Also, since IOP measured with the pneumatonometer (*Bhan et al.,* 2002; *Tonnu et al.,* 2005) increases approximately 0.40 mm Hg for every 10-µm increase in CCT, and the black participants had thinner corneas than the white participants had, the IOP could have been slightly underestimated.

In summary,

1) African/African-Caribbean patients with POAG or OHT have baseline aqueous humor dynamics similar to those of white Caucasians patients. Subjects with African ancestry have thinner corneas that may have masked some of the potential aqueous dynamics differences between the studied racial groups.

2) SLT lowers IOP by increasing the trabecular outflow but has no statistically significant effect on the aqueous production rate. To assess whether SLT and ALT have the same effect on aqueous humor dynamics, a comparative study between both lasers would be necessary.

CONCLUSIONS

Related to Main Objectives

1) There were no differences in any of the aqueous dynamics parameters between African/African-Caribbean and white Caucasians patients with treatment naive POAG and OHT.

2) SLT lowers IOP by increasing trabecular outflow in patients with POAG and OHT without an increase in uveoscleral outflow.

Related to Secondary Objectives

3) Age did not correlate with any of the aqueous dynamics parameters.

4) No significant differences in any of the aqueous dynamics parameters were found between PAOG and OHT. Eyes with POAG and OHT had reduced outflow facility and higher IOP compared to healthy eyes.

5) Outflow facility and IOP were inversely related in patients with PAOG and OHT.

6) No differences in AXL or ACD were found between African/African-Caribbean and white Caucasians patients. Nevertheless, subjects with African ancestry had thinner corneas than white Caucasians that may have masked some of the potential aqueous dynamics differences between the studied racial groups.

EPILOGUE

The results of this Thesis help to understand how SLT decreases IOP since we confirmed that SLT enhances trabecular outflow facility. Nevertheless, we were not able to provide insight into the greater risk for glaucoma in individuals of African descent as we were unable to find African descent individuals affected of POAG had different aqueous dynamics than white-Caucasians individuals. Older age, lower central corneal thickness, decreased corneal hysteresis, elevated IOP, myopia and positive family history are risk factors associated with POAG in individuals of African descent. Future studies should concentrate on the genetic component of POAG and potential differences in the structure of the lamina cribrosa that could be associated with a greater susceptibility to optic nerve damage.

ANNEX

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Glaucoma

Comparative Human Aqueous Dynamics Study between Black and White Subjects with Glaucoma

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Purpose. To compare the baseline aqueous humor dynamics in white Caucasians and patients of African origin with previously untreated primary open-angle glaucoma (POAG) or ocular hypertension (OHT).

Methods. Ninety-one participants were enrolled in this prospective, observational controlled study: 34 black subjects with POAG or OHT, 32 white Caucasian participants with POAG or OHT, and 12 black and 13 white healthy volunteers as the controls. All aqueous humor parameters were taken between 9 AM and 12 noon on the same day. Intraocular pressure (IOP) was measured by pneumatonometer; morning aqueous humor flow rate was measured by fluorophotometry and trabecular outflow facility by electronic Schiøtz tonography. Uveoscleral outflow was calculated by using Goldmann's equation with assumed episcleral venous pressure of 8, 9, 10, and 11 mm Hg. Differences among groups were analyzed with parametric and nonparametric tests and the relationship between aqueous dynamics parameters were evaluated with linear regression analyses.

Results. The POAG/OHT groups had similar IOP (white, 24.6 ± 3.0 mm Hg; black, 24.3 ± 4.0 mm Hg; comparison by Holm's sequential Bonferroni method (HBonf): $P_{HBonf} = 0.51$), outflow facility (white, 0.13 ± 0.09 μL/min/mm Hg; black, 0.13 ± 0.07 μL/min/mm Hg; $P_{HBonf} = 0.87$), aqueous flow (white, 2.36 ± 0.63 μL/min; black, 2.35 ± 0.53 μL/min; $P_{HBonf} = 0.95$), and uveoscleral outflow (white, 0.42 ± 1.59 μL/min; black, 0.58 ± 1.17 μL/min; $P_{HBonf} = 1.78$). POAG/OHT groups had significantly higher IOP and lower outflow facility than their healthy counterparts ($P < 0.01$). Black participants had significant thinner corneas (540 ± 37 μm vs. 564 ± 36 μm) than those of white participants ($P = 0.002$).

Conclusions. The aqueous humor dynamics of black African and white Caucasian patients with POAG or OHT have no significant differences. However, the significantly thinner corneas of the black patients may be masking potential differences in outflow facility and IOP measurements between the racial groups. (*Invest Ophthalmol Vis Sci.* 2011;52:9425–9430) DOI: 10.1167/iovs.10-7130

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Primary open-angle glaucoma (POAG) is four to eight times more prevalent in black Africans and African-Caribbeans than in whites.[1–4] Several studies have demonstrated differences in structural and biometric parameters and risk factors for glaucoma development among ethnic groups. Black subjects have thinner corneas[5–8] and larger optic discs[9–11] and may have thinner retinal nerve fiber layers (RNFLs).[12] The literature however, provides few insights into the potential racial differences in aqueous humor dynamics. Some studies on intraocular pressure (IOP) have reported higher IOP levels in blacks than in whites,[3,13,14] whereas others have found no differences[15] or even lower IOPs in black subjects.[16] Since the aqueous dynamics in this population have not been studied, we wanted to compare the baseline aqueous humor dynamics parameters in patients of African origin and white Caucasians with previously untreated POAG or ocular hypertension (OHT).

Methods

Ethics approval for this study was obtained from the St. Thomas's local research ethics committee. This research followed the tenets of the Declaration of Helsinki. Consecutive new patients referred to the glaucoma clinic were invited to participate. A patient information leaflet was provided at the initial contact, and signed consent was obtained before the measurements and treatment.

Participants enrolled in this prospective, observational, controlled study were divided into four groups: (1) white subjects with POAG or OHT, (2) black subjects with POAG or OHT, and (3) white healthy ocular normotensive (ONT) volunteers and (4) black healthy ONT volunteers as control groups.

Inclusion and Exclusion Criteria

Inclusion criteria were newly diagnosed, previously untreated adult POAG patients or patients with OHT that did or did not require treatment, both with IOP > 21 mm Hg at the screening visit. Glaucoma was diagnosed based on abnormal visual field testing and corresponding disc changes once seen by a fellowship-trained glaucoma specialist. Healthy volunteers had no ocular problems (other than refractive error) and IOP at screening <21 mm Hg. Exclusion criteria were secondary glaucomas including pigment dispersion syndrome and pseudoexfoliation, normotensive glaucoma, primary angle closure, history of uveitis, ocular trauma, intraocular or keratorefractive surgery, use of systemic medication that may affect aqueous humor production such as β-blockers, history of allergy or hypersensibility to fluorescein, and any abnormalities preventing reliable IOP or fluorophotometric readings.

Measurements

All patients underwent a clinical ophthalmic examination including visual acuity, slit lamp examination, gonioscopy, anterior chamber depth, and axial length (IOL Master; Carl Zeiss Meditec Inc., Dublin, CA), central corneal thickness (CCT; Pachmate DGH 55, DGH Technology, Inc., Exton, PA), visual fields (Humphrey automated white-on-white, 24-2 SITA-standard; Carl Zeiss Meditec), and dilated funduscopy.

The night before (10 PM) the fluorophotometric scans, participants self-administered from 3 to 6 drops of fluorescein sodium 2% (Minims; Bausch & Lomb, Kingston-upon-Thames, UK) topically into both eyes at 5-minute intervals depending on their ages (age, ≤25 years, 5 to 6 drops; age 26–35 years, 4 drops; >35 years of age, 3 drops).[17] Fluorophotometry was performed in both eyes with a scanning ocular fluorophotometer from 9 AM to 12 noon (FM-2, Fluorotron Master ocular fluorophotometer; OcuMetrics, Mountain View, CA). The aqueous flow rate was determined using dedicated software provided with the fluorophotometer (Appendix). Duplicate or triplicate scans were collected and repeated at 1-hour intervals for four measurements to determine the aqueous flow rate (F_t). Following each set of scans, IOP was measured using pneumotonometry (Model 30 Classic; Reichert Ophthalmic Instruments, Depew, NY); IOP was recorded as the arithmetic mean of a total of 12 measurements per eye: 3 measurements every hour alternating between eyes. Patients with IOP >21 mm Hg on the screening day may have had IOP of 21 mm Hg or less thereafter.

Tonographic outflow facility (C) was performed with an electronic Schiøtz tonographer (model 720; Berkeley Bioengineering, Inc., San Leandro, CA) at 10 AM. The facility of outflow was measured from the rate of decay of IOP in the supine position during application of a recording Schiøtz tonometer over a period of 4 minutes with a standard 5.5-g weight.[18] The R values of the curve at every 30-second time point were manually entered into the McLaren tonography computer program.[19] The program fits a second-degree polynomial by least-squares to the nine data points and determines by extrapolation the best-fit values for time 0 and time 4 minutes.

Uveoscleral outflow was calculated using Goldmann's equation[20] with an assumed episcleral venous pressure of 8, 9, 10, or 11 mm Hg.[19,21] F_t is the rate of aqueous humor formation, C is the tonographic facility of outflow, IOP is the intraocular pressure, P_v is the episcleral venous pressure, and F_u is the uveoscleral outflow.

$$F_t = C\,(\text{IOP} - P_v) + F_u$$

$$F_u = F_t - C\,(\text{IOP} - P_v)$$

Only one randomly (Excel random number generator; Microsoft, Redmond, WA) chosen eye per participant was included in the data analysis, when both eyes fulfilled the inclusion criteria.

Data Collection and Outcome Measures

Data including age, sex, race, IOP, tonographic outflow facility, aqueous flow rate, CCT, axial length, anterior chamber depth, cup-to-disc ratio, mean deviation in visual field testing and amount of fluorescein drops and time of instillation were recorded. Outcomes measures were outflow facility, IOP, aqueous flow rate, and uveoscleral outflow.

Data Analysis

Histograms and a Shapiro-Wilk test were performed to test for normality of distribution of data. A Shapiro-Wilk $W > 0.05$ was evidence of normal distribution. Student's t-test and one-way analysis of variance (ANOVA) were used to compare continuous variables among groups. When data did not follow normality, nonparametric methods of analysis (Mann-Whitney U and Kruskal-Wallis tests) were used. Post hoc comparisons among groups were made when appropriate, by Holm's sequential Bonferroni method (HBonf). The 95% confidence intervals (CI) for the mean and median difference between pairs for each outcome measure were calculated. The median difference 95% CIs were estimated using Hodges-Lehman methodology. Linear regression analyses were used to determine the correlation of one parameter versus another parameter of aqueous humor dynamics and the correlation of age versus each parameter. Fisher's exact test was used to compare the number of patients with POAG and OHT in each racial group. $P < 0.05$ was considered statistically significant (all analyses, SPSS 16.0; SPSS, Chicago, IL).

Results

One hundred and one patients with OHT/POAG and 32 healthy volunteers were recruited into the study. Only 66 affected subjects and 25 controls were included in the data analysis. Poor-quality Schiøtz tonography tracings ($n = 14$), with excess noise or extreme excursions, or poor fluorophotometric scans ($n = 27$), with extremely high or low baseline corneal fluorescein concentration, were identified in 41 participants and were therefore excluded from the analysis. Another participant was excluded for not having slept the night before the measurements were taken.[22]

In the black POAG/OHT group, 13 (38.2%) subjects had OHT and 21 (61.8%) had POAG, compared with 18 (56.2%) subjects with OHT and 14 (43.8%) subjects with POAG in the white affected group ($P = 0.21$).

The baseline characteristics from each group are shown in Table 1. The mean age of the study population was 58.7 ± 13.0 years. Age did not correlate with the outcome measures in any of the affected groups or in all participants combined (IOP, $P = 0.58$; C, $P = 0.08$; F_t, $P = 0.56$; and Fu_{10}, $P = 0.06$).

Subjects with POAG/OHT exhibited significantly higher IOP than their control counterparts ($P < 0.001$), but the black and white affected groups had comparable IOP ($P = 0.51$; 95% CI, −1.0 to 2.0). Black POAG/OHT subjects had significant thinner corneas than did the white POAG/OHT group ($P_{HBonf} = 0.009$; 95% CI, 8–43) but no other difference regarding CCT was observed between the rest of the groups. The mean CCT of all black participants was 540 ± 37 μm compared with 564 ± 36 μm in all white participants together (Table 2; $P = 0.002$; 95% CI, 8–40). CCT did not correlate significantly with any of the aqueous dynamics parameters.

Both the black and white groups with POAG/OHT had significantly lower outflow facility than their matched control group (black, $P_{HBonf} < 0.001$; 95% CI, −0.11 to −0.02; white, $P_{HBonf} < 0.001$; 95% CI, −0,17 to −0.03), but no differences were found between the affected groups ($P_{HBonf} = 0.51$; 95% CI, −0.03 to 0.04). A weakly significant inverse relationship was observed between outflow facility and IOP, with a reduction of 0.008 ± 0.002 μL/min/mm Hg ($R^2 = 0.16$; $P < 0.001$; Fig. 1). This relationship remained after adjustment for age ($R^2 = 0.21$; $P < 0.001$). Facility of outflow appeared not to be linearly correlated to the stage of glaucoma damage measured by mean deviation (MD) in visual field testing ($R^2 = 0.002$; $P = 0.72$) and cup-to-disc ratio ($R^2 = 0.007$; $P = 0.51$).

The mean aqueous flow rates for each group ranged from 1.81 to 2.36 μL/min. The black control subjects had significantly lower aqueous production than did the black affected subjects ($P_{HBonf} = 0.01$; 95% CI, 0.17–0.90). The aqueous production did not differ significantly between the black and white affected groups ($P_{HBonf} = 0.95$; 95% CI, −0.29 to 0.30). Aqueous flow was independent of IOP ($R^2 = 0.03$; $P = 0.07$).

The uveoscleral outflow was not different among groups, regardless of the assumed episcleral venous pressure. The 95% CI for the difference between the affected group medians for Fu_{10} ($P_v = 10$ mm Hg) was −0.74 to 0.58 μL/min. Affected patients, independent of their racial origin ($n = 66$), had a mean Fu_{10} of 0.75 ± 1.36 μL/min (median, 0.50; range, −4.04 to 2.68) compared with 0.47 ± 0.95 μL/min (median, 0.52; range, −1.51 to 2.19; $P = 0.51$) in all control subjects ($n = 25$). Contrary to outflow facility, the relationship between uveoscleral outflow (Fu_{10}) and IOP ($R^2 = 0.007$; $P = 0.44$) did not reach significance. Uveoscleral outflow and stage of glaucomatous damage were not related. Similar results were obtained with assumed episcleral venous pressures of 8, 9, and 11 mm Hg (Table 1).

TABLE 1. Comparison of Baseline Aqueous Dynamics Parameters in Patients with POAG or OHT and Healthy Volunteers

	POAG/OHT						Controls					
	Black (n = 34)			White (n = 32)			Black (n = 12)			White (n = 13)		
Parameters	Median (Mean)	IQR (SD)	Range (Min, Max)	Median (Mean)	IQR (SD)	Range (Min, Max)	Median (Mean)	IQR (SD)	Range (Min, Max)	Median (Mean)	IQR (SD)	Range (Min, Max)	P
IOP, mm Hg	23.51	14.01	20.10	25.10	14.10	11.20	19.10	14.18	28.20	17.10	23.10	10.10	<0.001*
	(24.13)	(41.01)	(−19.0, 39.0)	(24.16)	(13.20)	(−20.0, 31.0)	(18.11)	(12.27)	(−14.0, 22.0)	(17.10)	(12.18)	(−11.0, 21.0)	
F_r, μL/min	2.36	0.94	2.37	2.26	0.78	2.67	1.65	0.55	1.94	2.21	0.44	1.82	0.02†
	(2.35)	(0.53)	(1.32, 3.69)	(2.36)	(0.63)	(1.02, 3.69)	(1.81)	(0.52)	(1, 163.10)	(2.19)	(0.47)	(1.11, 2.93)	
C, μL/min/mm Hg	0.12	0.08	0.36	0.11	0.13	0.41	0.18	0.08	0.28	0.21	0.19	0.33	0.003*
	(0.13)	(0.07)	(0.03, 0.39)	(0.13)	(0.09)	(0.03, 0.44)	(0.18)	(0.07)	(0.07, 0.35)	(0.23)	(0.10)	(0.08, 0.41)	
Fu_8, μL/min	0.46	1.66	6.17	0.54	2.61	7.48	0.13	1.20	2.88	0.45	1.96	4.26	0.67*
	(0.32)	(1.29)	(−4.00, 2.17)	(0.15)	(1.73)	(−4.92, 2.56)	(−0.03)	(0.82)	(−1.92, 0.96)	(0.12)	(1.26)	(−2.33, 1.93)	
Fu_9, μL/min	0.61	1.53	5.92	0.63	2.43	7.10	0.31	1.22	2.77	0.60	1.82	3.98	0.76*
	(0.45)	(1.23)	(−3.72, 2.20)	(0.29)	(1.64)	(−4.48, 2.62)	(0.15)	(0.79)	(−1.57, 1.20)	(0.35)	(1.18)	(−1.92, 2.06)	
Fu_{10}, μL/min	0.76	1.46	5.69	0.75	2.24	6.72	0.45	1.24	2.66	0.75	1.68	3.70	0.77*
	(0.58)	(1.17)	(−3.44, 2.25)	(0.42)	(1.59)	(−4.04, 2.68)	(0.34)	(0.76)	(−1.22, 1.44)	(0.58)	(1.11)	(−1.51, 2.19)	
Fu_{11}, μL/min	0.89	1.41	5.46	0.87	2.07	6.34	0.57	1.29	2.55	0.90	1.55	3.47	0.81*
	(0.71)	(1.12)	(−3.16, 2.30)	(0.56)	(1.48)	(−3.60, 2.74)	(0.53)	(0.74)	(−0.87, 1.68)	(0.82)	(1.02)	(−1.10, 2.37)	

IQR, interquartile range; Fu_{8-11}, uveoscleral outflow with an assumed episcleral venous pressure of 8, 9, 10, and 11 mm Hg.

* Kruskal-Wallis test.

† One-way ANOVA.

TABLE 2. Descriptive Parameters of Black and White Patients with POAG or OHT and Healthy Volunteers

	POAG/OHT						Controls					
	Black (n = 34)			White (n = 32)			Black (n = 12)			White (n = 13)			
Parameters	Median (Mean)	IQR (SD)	Range (Min, Max)	Median (Mean)	IQR (SD)	Range (Min, Max)	Median (Mean)	IQR (SD)	Range (Min, Max)	Median (Mean)	IQR (SD)	Range (Min , Max)	P
Age, y	60.0 (58.2)	23.6 (12.9)	48.2 (30.8, 79.1)	61.6 (61.8)	22.2 (13.2)	47.8 (40.3, 88.1)	49.6 (51.1)	8.1 (10.6)	36.7 (38.1, 74.8)	61.5 (59.8)	16.4 (13.2)	45.4 (33.8, 79.2)	0.11*
ACD, mm	3.14 (3.19)	0.59 (0.38)	1.40 (2.59, 3.99)	3.23 (3.30)	0.46 (0.30)	0.99 (2.89, 3.88)	3.25 (3.23)	0.78 (0.37)	1.02 (2.78, 3.80)	3.41 (3.32)	0.42 (0.32)	1.17 (2.55, 3.72)	0.48†
AXL, mm	23.52 (23.67)	0.99 (0.83)	3.72 (22.12, 25.84)	23.56 (24.00)	1.73 (1.33)	4.72 (22.48, 27.20)	23.27 (23.45)	1.37 (0.77)	2.09 (22.34, 24.43)	24.03 (24.00)	1.90 (1.04)	3.46 (22.29, 25.75)	0.68†
CCT, μm	548 (539)	55 (37)	168 (438, 606)	569 (565)	43 (32)	121 (513, 634)	540 (543)	48 (37)	145 (479, 624)	572 (563)	72 (48)	158 (483, 641)	0.04*

IQR, interquartile range; ACD, anterior chamber depth; AXL, axial length.
* Significant, according to one-way ANOVA.
† Kruskal-Wallis test.

Table 3 summarizes the aqueous dynamics parameters of the POAG and OHT subjects, which showed nonsignificant differences.

Despite the prestudy sample size calculation based on the results of another study,[19] post hoc analysis of our data showed that our study would be able to detect a difference of 10.2% in IOP, 9.7% in aqueous flow, and only 38% in outflow facility, with a power of 80% and α (two-sided) = 0.05, if those differences existed.

DISCUSSION

Previous aqueous dynamics studies have included black participants[23] but this is the first study that specifically compares the aqueous dynamics between Africans/African Caribbeans and white Caucasians. Despite the anatomic differences reported between black and white populations, this study found no significant differences in the aqueous dynamics among patients with POAG/OHT of both racial groups.

Despite the number of patients with POAG being higher in the black affected group than in the white affected group, this disproportion between both racial groups would not have interfered in our analysis, since OHT and POAG patients have comparable aqueous dynamics.[24]

Contrary to some studies,[3,13,14] we found black and white participants with POAG/OHT had similar IOP, but this is likely to be due to the bias in our sampling as patients with IOP higher than 35 mm Hg at the screening visit, according to our ethics committee recommendations, would have been excluded from the study, to avoid delaying the instauration of the medical treatment. Also, since IOP measured with the pneumatonometer[25,26] increases approximately 0.40 mm Hg for every 10-μm increase in CCT, and the black participants had thinner corneas than the white participants had, the IOP could have been slightly underestimated.

To measure the trabecular outflow facility, we used the classic electronic Schiøtz tonometer because compared to pneumotomography or fluorophotometry techniques, the Schiøtz tonometer gives an accurate assessment of outflow facility with less variability over time.[19] Rejection rate of 14 of 101 subjects due to poor-quality tonography is only slightly higher than that found in a previous study by the same researchers.[19]

Since Grant[18] and Becker[27] reported a reduction in outflow facility in patients with glaucoma many years ago, other groups have come to the same conclusion.[23,24] We found a similar reduction in the outflow facility in both affected racial groups without a significant increase in the aqueous production. The measurements performed with the Schiøtz tonometer, are affected by ocular rigidity.[28,29] Indentation tonography makes no compensation for individual variations in ocular rigidity. The stiffer the eye, the greater the force needed to indent the cornea and displace the aqueous. Corneal hysteresis (CH) and corneal resistance factor (CRF) are dynamic measurements of the viscoelastic properties of the cornea that have been found to be lower in subjects of black African descent than in whites.[16,30] Several studies have shown an increase in CH and CRF with increasing CCT.[16,31,32] The higher the CH, the longer the cornea takes to return to its original shape, implying greater stiffness. Therefore, higher outflow facility measurements could have been obtained in the black participants on the basis of decreased ocular rigidity rather than the true equality found among the groups. On the other hand, Pallikaris et al.[33] measured in vivo the rigidity coefficient in 79 living human eyes and found no statistically significant correlation between ocular-rigidity coefficient and CCT. They reported that despite the low power to detect that correlation, CCT may have an effect on the corneal biomechanics, but may have less impact in ocular rigidity which involves the whole eye and not only the cornea.

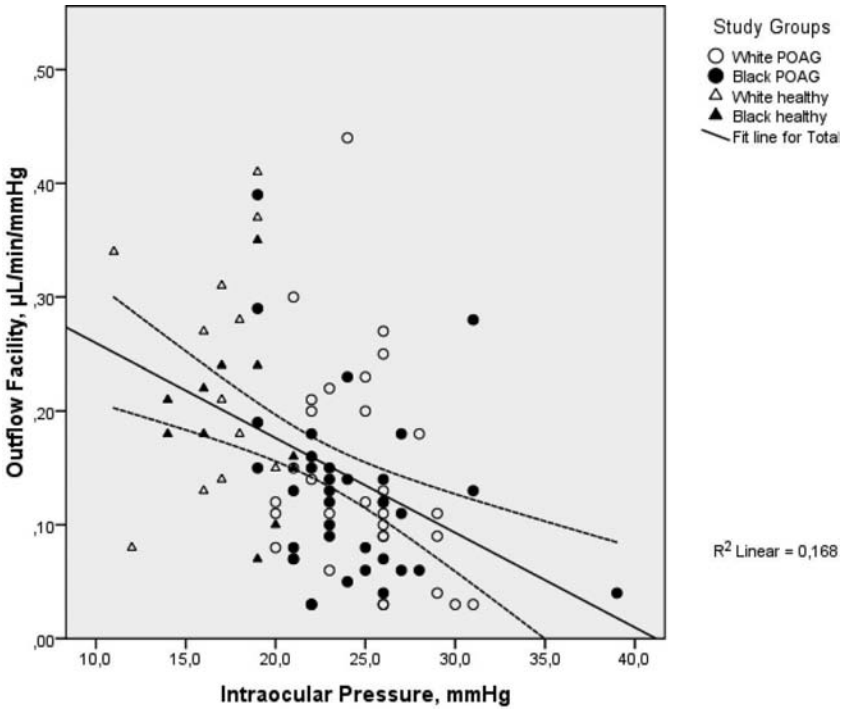

Figure 1. Scatterplot and bivariate regression analysis between the IOP and tonographic outflow facility (*C*). The regression line is $C = -0.008 \times \text{IOP} + 0.343$, $P < 0.001$, and $R^2 = 0.16$ (95% confidence bands are shown).

Larsson et al.[34] and Toris et al.[23] showed that the rate of aqueous production was not different in patients with glaucoma or ocular hypertension compared with healthy age-matched controls. This study confirmed this finding, and we also found no racial difference in the aqueous production rates.

The uveoscleral outflow was not significantly different between the white and black groups or the affected subjects and healthy volunteers, although it has been found to be reduced in beagle dogs with advanced glaucoma[35] and elevated in monkeys with laser-induced glaucoma.[36] In humans with POAG, Toris et al.[23] reported that uveoscleral outflow could be reduced in early stages of glaucoma, together with reduced trabecular outflow, but increases as the disease progresses to prevent the IOP from increasing further, but we did not find any differences between patients with OHT and POAG compared with normal subjects.

In summary, African/African-Caribbean patients with POAG or OHT have baseline aqueous humor dynamics similar to those of white Caucasians patients. Subjects with African ancestry have thinner corneas that may have masked some of the potential aqueous dynamics differences between the studied racial groups.

Appendix

The Yablonski Protocol of estimating aqueous humor flow rate was used.[37] Aqueous humor flow is the volume of aqueous humor produced by the ciliary body per unit of time.

$$F_t = K_o \cdot V_a$$

where, F_t is the aqueous humor flow (μL/min), V_a is the volume of the anterior chamber (μL), and K_o is the loss coefficient due to

Table 3. Comparison of Baseline Aqueous Dynamics Parameters between Patients with POAG Versus Those with OHT

	POAG (n = 35)			OHT (n = 31)			
	Median (Mean)	**IQR (SD)**	**Range (Min, Max)**	**Median (Mean)**	**IQR (SD)**	**Range (Min, Max)**	***P****
Age, y	62.7 (62.6)	19.7 (12.8)	55.0 (33.1, 88.1)	54.9 (56.9)	21.9 (12.9)	52.6 (30.8, 83.4)	0.07†
IOP, mm Hg	23.0 (23.7)	4.0 (2.8)	12 (19.0, 31.0)	26.0 (25.3)	6 (4.1)	20 (19,39.0)	0.07
F_t, μL/min	2.38 (2.40)	0.89 (0.58)	2.67 (1.02, 3.69)	2.18 (2.30)	0.81 (0.58)	2.49 (1.20, 3.69)	0.46†
C,μL/min/mmHg	0.12 (0.13)	0.07 (0.07)	0.41 (0.03, 0.44)	0.11 (0.13)	0.14 (0.09)	0.36 (0.03, 0.39)	0.98
Fu_8, μL/min	0.55 (0.38)	1.30 (1.39)	7.48 (−4.92, 2.56)	0.32 (0.07)	2.71 (1.63)	6.54 (−4.00, 2.54)	0.46
Fu_9, μL/min	0.67 (0.51)	1.28 (1.32)	7.10 (−4.48, 2.62)	0.43 (0.21)	2.45 (1.56)	6.30 (−3.72, 2.58)	0.46
Fu_{10}, μL/min	0.79 (0.64)	1.26 (1.25)	6.72 (−4.04, 2.68)	0.54 (0.35)	2.27 (1.49)	6.06 (−3.44, 2.62)	0.44
Fu_{11}, μL/min	0.92 (0.77)	1.27 (1.19)	6.34 (−3.60, 2.74)	0.89 (0.49)	2.09 (1.41)	5.82 (−3.16, 2.66)	0.40
CCT, μm	549 (547)	51 (39)	196 (438, 634)	554 (556)	50 (33)	129 (492, 621)	0.33†
MD, dB	−3.99 (−5.62)	5.22 (5.70)	30.52 (−29.97, 0.55)	−1.30 (−1.46)	2.24 (2.56)	15.53 (−7.58, 7.95)	<0.001

IQR, interquartile range; Fu_{8-11}, uveoscleral outflow with an assumed episcleral venous pressure of 8–11 mmHg.
* Mann-Whitney U test.
† Student *t*-tests.

bulk flow and diffusion from the anterior chamber (minutes^{-1}). K_o can be thought of as the fraction of anterior chamber volume cleared of fluorescein every minute, due to aqueous flow.

To calculate the aqueous flow, the program uses default variables that can be changed by the operator as necessary:

Corneal volume default value, 70 μL

Anterior chamber volume default value, 174 μL

CCT of each patient, in micrometers (We introduced each patient's corneal thickness instead of using the 500-μm default value to correct for the depth of the focal diamond. However, the corneal volume does not change if a different corneal central thickness is entered).

Once the relationship between cornea and anterior chamber concentrations of fluorescein becomes steady, the program can determine K_o and aqueous flow (F_l), as described in detail elsewhere.[37]

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ORIGINAL STUDY

The Effect of Selective Laser Trabeculoplasty on Aqueous Humor Dynamics in Patients With Ocular Hypertension and Primary Open-angle Glaucoma

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Purpose: To investigate the effect of primary selective laser trabeculoplasty (SLT) on outflow facility and aqueous flow rate in patients with primary open-angle glaucoma or ocular hypertension.

Methods: Eighteen eyes (9 with ocular hypertension and 9 with primary open-angle glaucoma) were included in this prospective noncontrolled study. Patients with intraocular pressures (IOPs) > 21 to 35 mm Hg were treated with 360-degree SLT after a baseline measurement of IOP, tonographic outflow facility, and morning aqueous humor production. Electronic Schiøtz tonography was used to measure the outflow facility. The aqueous flow rate was measured by fluorophotometry, and a pneumotonometer was used to measure the IOP. All measurements were repeated at least 3 months after the laser therapy. Paired Student *t* tests were used to compare aqueous dynamics parameters before and after treatment.

Results: The mean age of the study population was 56.7 ± 12.4 years. The IOP decreased significantly (21%) from 24.0 ± 3.0 to 18.9 ± 2.7 mm Hg ($P < 0.001$), whereas tonographic outflow facility increased significantly (55.5%) from 0.09 ± 0.05 to 0.14 ± 0.08 μL/min/mm Hg ($P = 0.003$) 3 months after laser treatment. No statistically significant changes in the production of aqueous humor were found ($P = 0.46$).

Conclusions: Our results show that SLT lowers the IOP by increasing the outflow through the trabecular meshwork, but it has no significant effect on the aqueous flow rate.

Key Words: trabeculoplasty, glaucoma, aqueous humor, outflow, intraocular pressure

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Selective laser trabeculoplasty (SLT) is used as a primary and adjunctive treatment to reduce the intraocular pressure (IOP) in patients with open-angle glaucoma and ocular hypertension (OHT).[1] SLT was developed by Latina in 1995 and was approved by the Food and Drug Administration in 2001.[2,3] It delivers over 100 times less energy than argon laser trabeculoplasty (ALT) while providing a similar IOP-lowering effect.[4–7] SLT uses a frequency-doubled, Q-switched, Nd:YAG laser and delivers a 400-μm-diameter treatment spot in 3 nanoseconds. The power ranges between 0.4 and 1.2 mJ with up to 120 applications per eye. Contrary to ALT, which causes visible thermal damage to the trabecular meshwork, SLT selectively targets the pigmented trabecular cells without damaging the adjacent nonpigmented meshwork structures.[8,9]

Although the exact mechanism of action of SLT remains unclear, a previous study undertaken in our Department showed that SLT reduces IOP by increasing trabecular outflow facility in patients with untreated primary open-angle glaucoma (POAG) and OHT.[10] We demonstrated a 29% reduction in IOP with only a 37.5% increase in outflow. The increase in outflow facility, although could not explain fully the observed reduction in IOP, suggests that SLT could also affect other aqueous dynamics parameters, such as aqueous production, uveoscleral outflow, or episcleral venous pressure. To elucidate whether SLT also affects the aqueous flow rate, we conducted this prospective noncontrolled observational study.

METHODS

The patients were recruited from our prospective aqueous dynamics study.[11] These were all newly diagnosed patients with POAG or OHT who were free to choose between prostaglandins or SLT treatment. Patients were invited to have, after baseline aqueous dynamics measurements, a second set of aqueous dynamics measurements 3 months after SLT treatment. Ethics approval for this study was obtained from the St Thomas's local research ethics committee. This research followed the tenets of the Declaration of Helsinki. A patient information leaflet was provided at the initial contact, and signed informed consent obtained before the baseline measurements and treatment.

Inclusion and Exclusion Criteria

Inclusion criteria were newly diagnosed, previously untreated adult POAG or high-risk OHT patients who required treatment, both with IOP > 21 mm Hg at the screening visit. Glaucoma was diagnosed by a fellowship-trained glaucoma specialist based on abnormal visual field testing and corresponding disc changes. Exclusion criteria were secondary glaucomas, normotensive glaucoma, primary angle closure, history of uveitis, ocular trauma, intraocular or keratorefractive surgery, advanced POAG (cup disc ratio ≥ 0.9 and/or visual acuity < 6/36), very high IOP (> 35 mm Hg), 1-eyed patients, use of systemic medication that may affect aqueous humor production such as β-blockers, history of allergy or hypersensitivity to fluorescein, and any

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abnormalities preventing reliable IOP or fluorophotometric readings. Patients with unreliable fluorophotometric scans and/or tonography tracings in either one of the 2 sets of measurements were also excluded. Only 1 eye randomly (Excel random number generator) chosen from each patient was included in the data analysis when both eyes fulfilled the inclusion criteria.

Measurements

At the screening visit, all patients underwent a clinical ophthalmological examination including visual acuity, slit-lamp examination, gonioscopy, anterior chamber depth and axial length (IOL Master; Carl Zeiss Meditec Inc., Dublin, CA) central corneal thickness (Pachmate DGH 55; DGH Technology Inc., Exton, PA), IOP measured by Goldmann applanation tonometry, visual field tests (Humphrey automated white on white, 24-2 SITA standard), and dilated fundoscopy.

All patients had IOP, outflow facility, and aqueous flow rate measurements performed on the day of treatment and at least 3 months after the SLT treatment. If additional glaucoma medical treatment was started to achieve a target pressure within that interval, patients were excluded from the study. The night before (10 PM) the fluorophotometric scans, participants self-administered from 3 to 6 drops of fluorescein sodium 2% (Minims; Bausch & Lomb, UK) topically into both eyes at 5-minute intervals depending on their age (aged 25 y or younger, 5 to 6 drops; aged 26 to 35 y, 4 drops; and above 35 y of age, 3 drops).[12] Fluorophotometry was performed using a scanning ocular fluorophotometer from 9 AM to 12 PM (FM-2, Fluorotron Master Ocular Fluorophotometer; OcuMetrics, Mountain View, CA). Duplicate or triplicate scans were collected and repeated at 1-hour intervals for 4 measurements to determine the aqueous flow rate (Ft). After each set of scans, IOP was measured using pneumotonometry (Model 30 Classic; Reichert Ophthalmic Instruments, Depew, NY); IOP was recorded as the arithmetic mean of a total of 12 measurements per eye: 3 measurements every hour alternating between eyes. Patients with IOP > 21 mm Hg on the screening day may have had IOP of 21 mm Hg or less thereafter.

Tonographic outflow facility (C) was performed using an electronic Schiøtz tonographer (Model 720; Berkeley Bioengineering Inc., USA) between 9 AM and 12 PM. The facility of outflow was measured from the rate of decay of IOP in the supine position during application of a recording Schiøtz tonometer, over a period of 4 minutes with a standard 5.5 g weight.[13] The "R" values of the curve at every 30-second time point were manually entered into the McLaren tonography computer program. The program fits a second-degree polynomial by least squares to the 9 data points and determines the best-fit values for time 0 and time 4 minutes by extrapolation.

Treatment

All patients underwent 360-degree SLT treatments that were performed by Adanna Obi, Rahat Husain, and Kin-Sheng Lim. Pilocarpine 2% to 4% drops (Minims, Pilocarpine nitrate; Chauvin Pharmaceuticals Ltd, Surrey, UK) were instilled half an hour before SLT. SLT treatment was performed using the Ellex Solo machine (Ellex, Adelaide, Australia), spot size of 400 μm, duration 3 nanoseconds. Starting energy level was 0.6 mJ. The energy level was titrated at the 3-o'clock position up to the point where champagne bubbles or minimal blanching was visible. A total of 90 to 100 laser burns were given using the Magna view gonioscopy lens (Ocular Instruments, USA) to visualize the trabecular meshwork. Apraclonidine hydrochloride 1% drops (Iopidine 1%; Laboratòires Alcon S.A., Kayserberg, France) were instilled before and after laser therapy to prevent the IOP spike after laser therapy. All patients had a standardized postoperative regimen of dexamethasone 0.1% eye drops (Maxidex; Alcon Laboratories, UK) 4 times a day for 5 days to treat intraocular inflammation after SLT.

Data Collection and Outcome Measures

Data including age, IOP, tonographic outflow facility, aqueous flow rate, central corneal thickness, axial length, anterior chamber depth, and amount of fluorescein drops and time of instillation were recorded. The outcome measures were outflow facility, IOP, and aqueous flow rate.

Data Analysis

The data were analyzed by statistical software SPSS 16.0. A Shapiro Wilk W value of >0.05 was evidence of normal distribution. Paired Student t tests were used to compare aqueous dynamics parameters before and after treatment. A P value <0.05 was considered statistically significant.

RESULTS

Thirty consecutive patients with OHT/POAG treated with 360-degree SLT and baseline and posttreatment aqueous dynamics data were identified from our database. Poor-quality Schiøtz tonography tracings (n = 6), with excess noise or extreme excursions, and poor fluorophotometric scans (n = 5), with extremely high or low baseline corneal fluorescein concentration, were identified in 11 eyes and were therefore excluded from the analysis. One patient was advised to use antihypertensive drops after SLT and had to be excluded from the study. Eighteen eyes (9 OHT and 9 POAG) with a median follow-up time post-SLT of 3.6 (2.8 to 11.0) months were included in the final analysis. The mean age of the study population was 56.7 ± 12.4 (30.8 to 79.3) years. Fourteen patients were male. No significant sex differences in prelaser and postlaser aqueous dynamics parameters were found. The SLT treatment was well tolerated and no postlaser complications were observed. The mean total energy used was 95.5 ± 17.6 mJ (62 to 128 mJ; n = 14).

All study parameters were normally distributed. Differences between aqueous dynamics parameters at baseline and after SLT treatment are shown in Table 1. A statistically significant reduction in IOP of 5.0 ± 3.4 mm Hg (21%) with a significant increase in trabecular outflow facility of 0.05 ± 0.06 μL/min/mm Hg (55.5%) after SLT was observed. Trabecular outflow facility change after SLT (−0.05 to 0.24 μL/min/mm Hg) and change in IOP from baseline were not significantly correlated (Spearman $\rho = 0.25$, $P = 0.30$). No significant change in aqueous flow rate was observed.

DISCUSSION

This study investigates the effect of SLT on aqueous dynamics in treatment of naive subjects with newly diagnosed POAG and OHT. We found a significant reduction in IOP with an associated increase in tonographic outflow

TABLE 1. Comparison of Aqueous Dynamics Parameters Before and After 360-degree SLT Treatment

	Before SLT Treatment (n = 18)			After SLT Treatment (n = 18)			
Parameters	**Mean**	**SD**	**Range (min, max)**	**Mean**	**SD**	**Range (min, max)**	***P***
IOP (mm Hg)	24.0	3.0	12.2 (17.0, 29.2)	18.9	2.7	10.2 (15.2, 25.5)	< 0.001*
Ft (μL/min)	2.24	0.83	2.69 (0.99, 3.69)	2.09	0.70	2.27 (1.10, 3.37)	0.46
C μL/min/mm Hg	0.09	0.05	0.18 (0.02, 0.20)	0.14	0.08	0.27 (0.03, 0.30)	0.003*

*Statistically significant with a $P < 0.05$.
C indicates trabecular outflow facility; Ft, aqueous flow rate; IOP, intraocular pressure; n, number of eyes; SLT, selective laser trabeculoplasty.

facility 3.6 months after SLT with no change in aqueous production.

Overall, we found a 21% reduction in IOP that is comparable with previous SLT and ALT studies.[10,14–16] The IOP-lowering effect of SLT was accompanied by a 55.5% increase in tonographic outflow facility. Although the increase in facility of outflow is greater than we previously reported, it is similar to other studies on the effect of ALT on aqueous dynamics.[15–17] However, as highlighted by Goyal et al,[10] those studies were mainly done in medically treated patients, making any direct comparison difficult.

Bergeå and Svedbergh[17] reported a 33% reduction in IOP with a tonographic outflow facility increase of 63.5% in medically untreated eyes (n = 11) with POAG. However, their patients had higher baseline IOP and had only 180-degree ALT. Brubaker and Liesegang's study[15] found similar IOP reduction (29%) and increase in tonographic outflow facility (64%) after ALT in medically treated patients (n = 17) using the same tonographic technique. They did not find any effect of ALT on aqueous production. Another study by Thomas et al[16] obtained the same reduction in IOP (29%) and increase in outflow facility (64%) as Brubaker and Liesegang but in patients with different types of glaucoma who were on maximal-tolerated medical treatment. Despite the patients in those studies being already on medication before the laser treatment, they had similar baseline IOP as our previously untreated patients. Topical medications do not seem to affect SLT success. However, SLT efficacy has been positively associated with baseline IOP.[18,19] Thus, regardless of whether patients are medically treated before the laser treatment, equivalent results would be obtained if patients had similar IOP before the laser therapy.

Yablonski et al[20] studied the aqueous dynamics changes after ALT (n = 15) and found a 34% increase in tonographic outflow facility with no effect on the rate of aqueous humor production. They also observed a reduction in uveoscleral outflow that was attributed to the reduction in IOP after laser therapy.

We found that the rate of aqueous humor production was not affected by SLT. Our results suggest that the eventual mechanism of action of SLT is likely to be very similar to ALT. Both types of trabeculoplasty would reduce the IOP by increasing the outflow through the trabecular meshwork. Buskirk[21] suggested ALT may have a mechanical as well as a biochemical effect on the trabecular meshwork. Kramer and Noecker[8] compared the histopathologic changes in the trabecular meshwork after ALT and SLT in human eye bank eyes. They demonstrated that both types of laser altered the ultrastructure of the trabecular meshwork endothelial cells. However, ALT was also associated with coagulative damage to the trabecular beams. They suggested that SLT may only have a selective biological effect on the trabecular meshwork while ALT would have an additional mechanical effect, responsible for the fibrosis and scarring. If ALT and SLT have similar mechanisms of action, the structural damage produced by ALT may be an unnecessary consequence of the argon laser, not contributory to the trabeculoplasty IOP-lowering effect.[5] It is possible that immediately after the SLT, there may be some effect on the uveoscleral outflow secondary to the inflammatory response created by the laser treatment, which would subside after a few months. Unfortunately, because there are currently no reliable clinical methods of measuring uveoscleral outflow (Fu), this theory remains highly speculative at best.

Pilocarpine and apraclonidine drops instilled before SLT treatment would not have interfered with our measurements, as their effect on outflow facility and aqueous flow will at maximum last for a few days. Apraclonidine has been associated with an increase in fluorophotometric outflow facility,[22] but there is no evidence that α-agonists affect tonographic outflow facility using the Schiøtz technique as was used in this study.[23]

To our knowledge, this is the first study that investigates the effects of SLT on outflow facility and aqueous production, helping us to improve our understanding of its mechanism of action. To assess whether SLT and ALT have the same effect on aqueous humor dynamics, a comparative study between both lasers would be necessary.

In summary, SLT lowers IOP by increasing the trabecular outflow but has no statistically significant effect on the aqueous flow rate.

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