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The effect of ultrasound, phacoemulsification and uveitis on aqueous humour dynamics

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Thesis submitted to the King's College London in fulfilment of the requirements for the degree of Doctor of Medicine

October 2019

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Declaration

I, Pouya Alaghband, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Pouya Alaghband

Date 30th September 2019

Abstract

This thesis, presented as a thesis incorporating publications, examines the effects of phacoemulsification, uveitis and high intensity focused ultrasound on the aqueous humour dynamics.

The interplay between aqueous humour parameters are complex. The impacts of various pathologies and pharmacological changes in glaucoma and healthy subjects have been extensively investigated. However, the evidence on the modern cataract surgery and the aqueous humour dynamics has not been consistent. It has been shown in previous studies that phacoemulsification reduces intraocular pressure (IOP). But the mechanism through which IOP is reduced is not fully understood. I performed electronic Schiøtz tonography to measure outflow facility before and after phacoemulsification. I demonstrated that the tonographic outflow facility (TOF) enhancement as a result of cataract surgery in open angle glaucoma (OAG) cases is responsible for IOP reduction.

On the other hand, uveitis can be a sight threating condition especially due to its sequlae such as glaucoma. The animal studies on the effects of uveitis on the aqueous humour dynamics are limited. They are mainly focused on the acute phase of the intraocular inflammation. However, only two human studies in assessing the aqueous humour dynamics are available to date. But neither has been comprehensive nor comparative with healthy subjects or quiescent eyes. In my study of the uveitis and aqueous humour dynamics changes, I have

illustrated that raised IOP is mainly due to compromised TOF, whilst the-aqueous flow rate and the uveoscleral outflow remained unchanged.

There is a plethora of new treatment modalities emerging in the field of glaucoma every day. One of these new additions is high intensity focused-ultrasound (HiFU). Although it reduces IOP to some extent the mechanism of action is unclear. There have been some speculations that it may affect the aqueous flow rate and the uveoscleral outflow. Also, there have been no studies on the effects of HiFU on the aqueous humour dynamics especially with glaucoma medication washout. In my study of the effect of HiFU on aqueous humour dynamics, I have shown that IOP at three months post procedure was reduced by 12% whilst aqueous flow rate was reduced by 10%. The calculated uveoscleral outflow and TOF was remained unchanged.

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Publications arising from this thesis

Alaghband, P. et al. (2020) The effect of high intensity focused ultrasound on aqueous humour dynamics in glaucoma patients. Ophthalmology Glaucoma. 3(2), 122-129

Alaghband, P. et al. (2019) The aqueous humour dynamics in uveitic eyes. *American journal of ophthalmology*. 208, 348-355

Alaghband, P. et al. (2018) The effect of phacoemulsification on aqueous outflow facility. *British Journal of Ophthalmology*. 102, 1520–1526.

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Abbreviations

ACD. Anterior chamber depth
ANOVA. Analysis of variance
AS-OCT. anterior segment optical coherence tomography
AXL. Axial length
C. *Coefficeint of aqueous outflow facility*
CCT. Central corneal thickness
CI. Confidence interval
ECP. *endocyclophotocoagulation*
EVP. Episcleral venous pressure
FITC. *fluorescein isothiocyanate*
g. *gram*
GAG. Glycosaminoglycan
HiFU. *High intensity Focused Ultrasound*
Hz. Hertz
IOL. *Intraocular lens*
IOP. Intraocular pressure
JCT. *Juxta canalicular tissue*
LVDT. *Linear variable differntial transformer*
MD. *Mean deviation*
MTMT. maximum tolerated medical therapy
NHS. *National Health Service*
NOS. Nitric oxide
OAG. Open angle glaucoma
OHT. Ocular hypertension
OHTS. Ocular Hypertension Treatment Study
PAS. Peripheral anetrior synachiae
PDG. pigment dispersion glaucoma
POAG. Primary open angle glaucoma
SITA. Swedish thresholding interactive algorithm
SPSS. Statistical package for social sciences
TM. Trabecular meshwork
TOF. Tonographic outflow facility
U. Unconventional outflow
UCP. Ultrasound ciliary plasty
UV. ultraviolet
WTW. white-to-white

Chapter 1| Introduction

Aqueous humour dynamics has been studied extensively to clarify the mechanism of action of new glaucoma medications. Additionally, it has been utilised to understand the effects of more commonly performed ophthalmic procedures such as cataract surgery and laser trabeculoplasty. However, the results of studies in cataract surgery have been inconsistent. Therefore, the unmet need for elucidating the alterations of aqueous humour dynamics parameters exists.

Newer technologies harness novel techniques to treat glaucoma more effectively and more precisely. One example is high intensity focused ultrasound (HiFU). This treatment modality targets ciliary bodies in a titrated and more controlled manner by applying ultrasonic energy to the eye and specifically ciliary bodies to reduce aqueous flow rate. This subsequently reduces intraocular pressure (IOP). To date no studies have investigated the aqueous humour dynamics changes after cyclodestructive therapy.

Moreover, evaluating aqueous humour dynamics has enabled investigators to assess the effect of various systemic conditions (such as diabetes, ageing, etc.) and ocular conditions (including uveitis) on the aqueous humour dynamics parameters. There are only a few studies that have explored the changes of the-aqueous humour dynamics in a small number of patients with Fuchs heterochromic cyclitis (Johnson et al., 1983). However, in active uveitis (such as in Fuch's heterochromic cyclitis) the aqueous-blood barrier is broken and therefore, it poses a challenge in aqueous humour dynamics

measurements.-This is particularly crucial when fluorophotometry is utilised in assessing aqueous flow rate. Furthermore, to date there are no studies investigating uveitic glaucoma and comparing it with healthy subjects with hypotensive medication washout.

Consequently, we still lack understanding in some of the areas which have been highlighted. The basis of this thesis is to shed some light in some of these areas.

Chapter 2|Materials and methods

Aqueous humour dynamics

In this chapter, I will scrutinise the available evidence on each aqueous humour dynamics parameter and delve into the historical aspects of each parameter.

Glossary

- Aqueous humor in the history
- Aqueous humor flow rate
- Trabecular meshwork outflow (outflow facility)
- Unconventional outflow (uveoscleral outflow)
- Episcleral venous pressure

Aqueous humour in the history

Until the turn of the 20th century it was a common belief that aqueous humour is a stagnant fluid within the eye. Leber (Leber, 1903) described a model of aqueous humour dynamics which entailed that aqueous humour is produced in the posterior chamber and then traversed through the pupil into the anterior chamber. But then Seidel's work (Seidel, 1921) unravelled the mystery of the-aqueous humor dynamics further. He perfused the enucleated rabbit's eye with the indigo carmine dye from a reservoir which was attached to the eye. He observed the displacement of the dye, between the eye and the chamber. Depending on the height of the reservoir, the dye displaced towards or away from the reservoir. Then he increased the height of the reservoir and he noticed that the fluid is flowing through the tubing to the eye from the reservoir. Subsequently the dye flowed towards the episcleral veins and filled them. He deduced that aqueous humour is constantly made and replaced by fresh newly produced fluid. The origin of aqueous humour, however, was not elucidated at that time. Boerhavve (Boerhaave, 1771) was the first person to discover the existence of aqueous veins. Later Ascher (Ascher, 1949) demonstrated that episcleral veins contain aqueous humour and they have a role in removing the aqueous humour from the eye. Goldmann (Goldmann, 1950) administered the fluorescein systemically and observed that it first started to appear in the anterior chamber of the eye and then eventually in the episcleral veins.

Sir-Stewart Duke-Elder (Duke-Elder, 1932) suggested that the aqueous is the filtration of plasma. Jonas Friedenwald (Cogan et al., 1955) was the advocate of the notion that aqueous humour is the ultrafiltration of plasma. Over the years, the focus on the source of aqueous humour formation narrowed down further. At the beginning, the attention was on an organ (i.e. the eye) and later it was more focused towards sections of the eye (i.e. ciliary processes) and even more precisely pointed to the ciliary epithelium; Later it was even further drilled down to water channels called aquaporins (Yamaguchi et al., 2006) which actively work as a conduit for water in and out of cells.

We now know that the aqueous humour drains through the trabecular-meshwork, then the Schlemm's canal, collector channels, aqueous veins, episcleral veins and finally into the systemic venous system.

Different aqueous outflow pathways exist; conventional and unconventional. It is assumed that some of the fluid drained backwards into the vitreous and optic nerve; however, the amount of the flow is very negligible. The main unconventional pathway, however, is via the uveoscleral outflow. Overby (Johnson et al., 2017) and associates suggested some methods to measure this in the animal models. This pathway accounts for about 20% of the outflow. Most-of the outflow is through the trabecular meshwork (conventional pathway). Each aqueous humour parameter will be explained in more details in the following pages.

Aqueous humour flow rate

Several methods exist to measure the aqueous flow rate including direct and indirect techniques. The ideal method is repeatable, does not change the intraocular pressure (IOP) and it is indirect. I will explore each method separately in the following paragraphs.

Several direct methods were introduced during the 1950s. The principals were that the eye was cannulated and the infusion at a known rate and known pressure level were utilised and subsequently the drainage was measured directly (Brubaker and Riley, 1972; Brubaker and Worthen, 1973; Kaufman, 1979; Bill and Barany, 1966; Langham and Taylor, 1960; Langham and Rosenthal, 1966). However, these methods were invasive, and they were not feasible or ethical to be carried out on humans routinely. The next best option was indirect methods of testing. One of these methods was the systemic injection of fluorescein which was first described by Ehrlich (Ehrlich, 1882) approximately over a century ago. He administered fluorescein systemically in rabbits and then the fluorescein started to appear in the anterior chamber of the-eye. Lindner (Lindner, 1920) measured the amount of fluorescein in the-anterior chamber quantitatively. Amsler and Huber (Amsler and Huber, 1946) improved the existing technique and simplified it. Their method was utilised in other subsequent studies of assessing aqueous-blood barrier by other

researchers (Gaedertz and Wittgenstein, 1928; Lugossy, 1959). Over the past six decades, there has been several modifications to the fluorescein technique.

- Barany and Kinsey (Barany and Kinsey, 1949), created a method to measure aqueous flow rate using the disappearance of fluorescein from the anterior chamber.
- In 1950s, Goldmann (Goldmann, 1950, 1951) enhanced the existing methodology and increased the accuracy of the measurement of fluorescein in a more quantitative fashion. In his method, Goldmann measured the amount of unbound fluorescein in plasma and anterior chamber after systemic administration of fluorescein. This was the first attempt to measure aqueous humor flow rate in humans quantitatively. Nevertheless, his technique was so complex that later not many investigators adopted Goldmann's technique.
- Langley and MacDonald (Langley and MacDonald, 1952) applied fluorescein topically and named their technique the "dye-dilution method". They proposed that the aqueous humour flow rate could be extrapolated from the rate of dilution of the aqueous. Later, Langham and Wybar (Langham and Wybar, 1954) devised the first slit lamp fluorophotometer.
- Holm and associates (Holm and Wiebert, 1968; Holm and Krakau, 1966; Holm, 1968) facilitated another technique called "pupillary bubble". In-this method, they applied fluorescein topically and observed the

enlargement of the clear aqueous fluid as it entered the anterior chamber, the fluorescein mixed with aqueous humour. However, from a technical point of view, it was challenging to measure the volume of aqueous as it expanded.

- Maurice (Maurice, 1963) explored the fluorescein tracer in the aqueous humour studies extensively. He had several reasons for choosing fluorescein as a suitable tracer (McLaren and Brubaker, 1985; McLaren, 2009; Brubaker, 1982). His reasons were as followed:
    - It is not metabolised by the eye and it could disappear through different paths
    - It penetrates via the epithelium of the cornea and washes out through the tear film
    - It penetrates through the limbus
    - It enters the endothelium and gains access to the aqueous humour

In the 1960s Maurice (Maurice, 1963) introduced an enhanced model of the-fluorophotometer with a significant reduction in the signal-noise ratio. Subsequently Jones and Maurice (Jones and Maurice, 1966) collaboratively devised an improved version of their original fluorophotometer which was convenient to use, and reproducible with the least ocular disturbance. Additionally, their method accurately measured the aqueous flow rate from a modified dye-dilution technique.

A decade later Yablonski (Yablonski et al., 1978), modified Jones and Maurice's "corneal depot" technique by applying fluorescein topically or through iontophoresis. Furthermore, this technique is not affected by the iris colour. The measurements would still be accurate and repeatable unlike the Jones and Maurice's method. Also, the drug induced changes of the aqueous flow rate were captured within a few hours by facilitating the Yablonski-protocol. These changes have been incorporated into the commercial fluorophotometer device software. The commercially available fluorophotometers operate based on the Waltman and Kaufman's slit lamp fluorophotometer (Waltman and Kaufman, 1970). This is based upon appearance and disappearance of fluorescein in the anterior chamber of the eye.

The beauty of utilising fluorescein in the aqueous humour flow rate measurement is to enable the investigators to assess the aqueous flow rate in an un-anaesthetised eye. This is more convenient and safer than other methods mentioned earlier. Additionally, topical application of fluorescein eliminates the risk of the systemic side effects of fluorescein administration including anaphylaxis reaction. In modified Jones and Maurice's "corneal depot" technique, it is crucial to saturate the cornea uniformly by using topical fluorescein a few hours (at least 8 hours) (waiting period) prior to the measurement of the aqueous humour flow rate determination (Brubaker, 1982; R. Brubaker, 2004; Brubaker and McLaren, 1985).

Currently two different types of fluorophotometers exist: slit lamp mounted and stand-alone scanning devices. The advantage of the former is a direct visualisation of the target which is useful in guiding the subjects to stabilise their eyes during measurement. But the downside is that the movements of the eye create artefacts. The issue with eye movement can be overcome in scanning fluorophotometers by encouraging the subjects to maintain their gaze at the light target (within the eyepiece of the device) during fluorophotometry.

The process commences on the previous night. The participants are instructed to self-administer 3 to 5 drops of high concentration of fluorescein sodium 2% (Minims; Bausch & Lomb, Kingston-upon-Thames, UK) topically into both eyes at 5-minute intervals at 10 pm, the night before the test (depending on their age (age<25 years, 5 drops; age 26–35 years, 4 drops; >35 years of age, 3 drops) (Brubaker et al., 2001). It has been noted that during the first 3 hours after the fluorescein application, the concentration of fluorescein in the cornea falls, while the concentration of fluorescein in the anterior chamber rises. After this preliminary stage, the fluorescein concentration reaches a steady state and then both fall in a very continuous and linear fashion. Some of the fluorescein penetrates the epithelium of the cornea (approximately under 500 ng). Then reaches the stroma and penetrates the endothelium and finally enters the anterior chamber. When the corneal stroma is saturated evenly, the concentration of fluorescein is measured in the cornea and the anterior chamber simultaneously.

The aqueous flow rate is estimated using the Jones and Maurice equation (Equation 1) (Jones and Maurice, 1966):

Equation 1-Jones and Maurice equation

$$Flow = \frac{-\Delta mt}{Ca\Delta t} - d$$

Δmt is the change in the total amount (mass) of fluorescein in the cornea and anterior chamber on ***Δt*** time, C_a is the mean concentration of fluorescein in the anterior chamber between each interval and ***d*** is the amount of fluorescein which is lost through diffusion into the vessels. ***mt*** is calculated from the formula below:

$$mt = vcCc + vaCa$$

In this equation, ***vc*** and ***va*** are the volumes of cornea and anterior chamber respectively and C_c and C_a is the concentration of fluorescein in the cornea and anterior chamber respectively (these could be calculated utilising Pentacam or using pre-assumed values).

Commercially available fluorophotometers were initially designed to detect blood-barrier breakdown in diabetic retinopathy cases in the early stages of the disease before the clinical signs become apparent (Plehwe et al., 1989).

Fluorophotometry for the purpose of aqueous humour flow rate measurement is performed in both eyes with a scanning ocular fluorophotometer from 9 AM to 12 PM (FM-2, Fluorotron Master ocular fluorophotometer; OcuMetrics,

Mountain View, CA). The Fluorotron system is made up of three major components (Figure 2-1).

- The electro-optic system (optic head) containing excitation and detection modules
- The computer
- The mechanical section

Figure 2-1 Shows Fluorotron machine

The optic head is a fluorophotometer which delivers a specifically focused excitation beam (440-480 nm) (from a halogen lamp) of blue light into the ocular cavity and then receives the resulting fluorescent green light and directs

it into a photon counting photomultiplier tube (531-634 nm). By changing the focal plane every 0.25 mm, as many as 149 sequential readings are made along an axis from a position posterior to the retina to a position anterior to the cornea. The operation of the optic head is controlled by the computer, prompting the operator through the entire procedure. The operator only aligns the eye and starts the scan. The scan is then automatically performed and displayed on the screen and printed in graphic and digital formats. Scan and patient data are stored electronically. The computer is used to process the data by using the appropriate program *(www.ocumetrics.com).*

Optic head features

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

The depth of resolution is the distance that a very large signal is reduced to 3%. Background noise in itself does not affect sensitivity as long as it is constant as it can be subtracted out. It is the fluctuation in background that can be problematic. That is why 3x the fluctuation in background is the limit to sensitivity.

Reproducibility is related to signal (very directly in a photon counting system like the Fluorotron), so the deviation in reproducibility is specified for lower concentrations versus higher concentrations.

Some of the fluorescence emitted in the eye is detected by the pick-up optics which only gather light coming from the focal plane of the illuminating beam. The intersection of the illuminating (excitation) beam and pick-up optical path defines a small area inside the anterior chamber called the "focal diamond" (Figure 2-2). This is the area where the fluorescence is detected by photodetectors. The device is fitted with two green filters in front of the photodetectors. This enables the detection of only light in the fluorescence bandwidth within the focal diamond. The measurement area is scanned, and the photodetectors count the number of photons emitted from the scanned area.

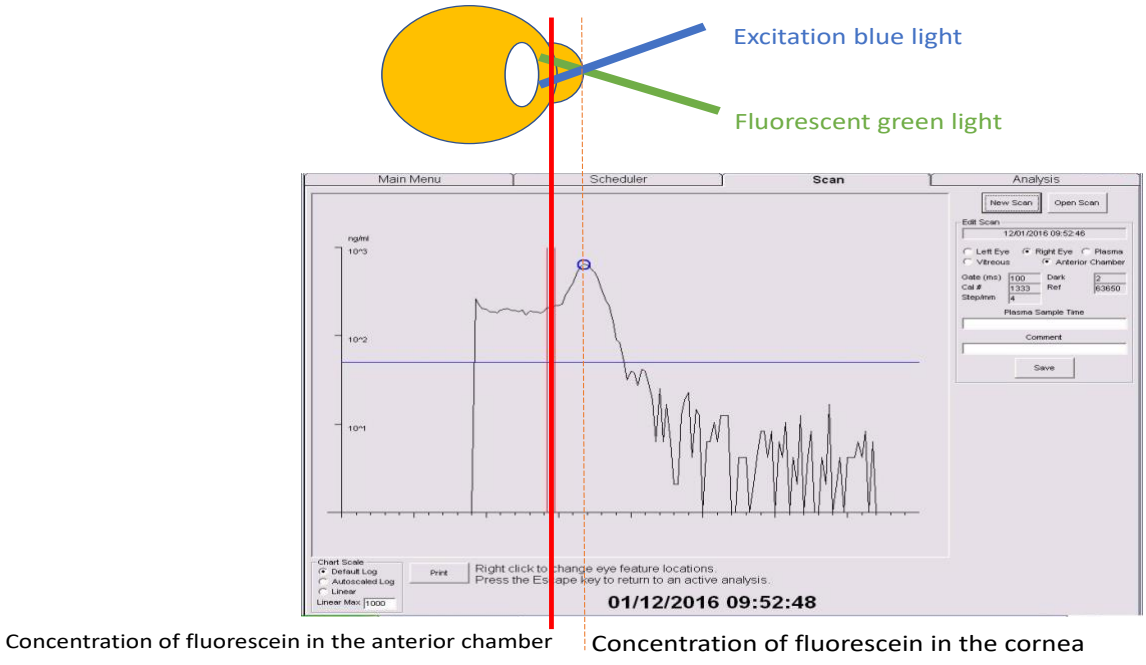


Figure 2-2. Depiction of excitation and fluorescence emitted lights and focal diamond (the intersection between the blue and green lights)

The aqueous humour flow rate is determined using dedicated software provided with the fluorophotometer. Triplicate scans are collected and repeated at hourly intervals for four measurements to determine the aqueous flow rate (*Ft*).

Aqueous humour flow rate is the volume of aqueous humour produced by the ciliary body per unit of time (Yablonski et al., 1978).

$$Ft = Ko\ x\ Va$$

where, ***Ft*** is the aqueous humour flow rate (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 clearance of fluorescein every minute, due to aqueous flow.

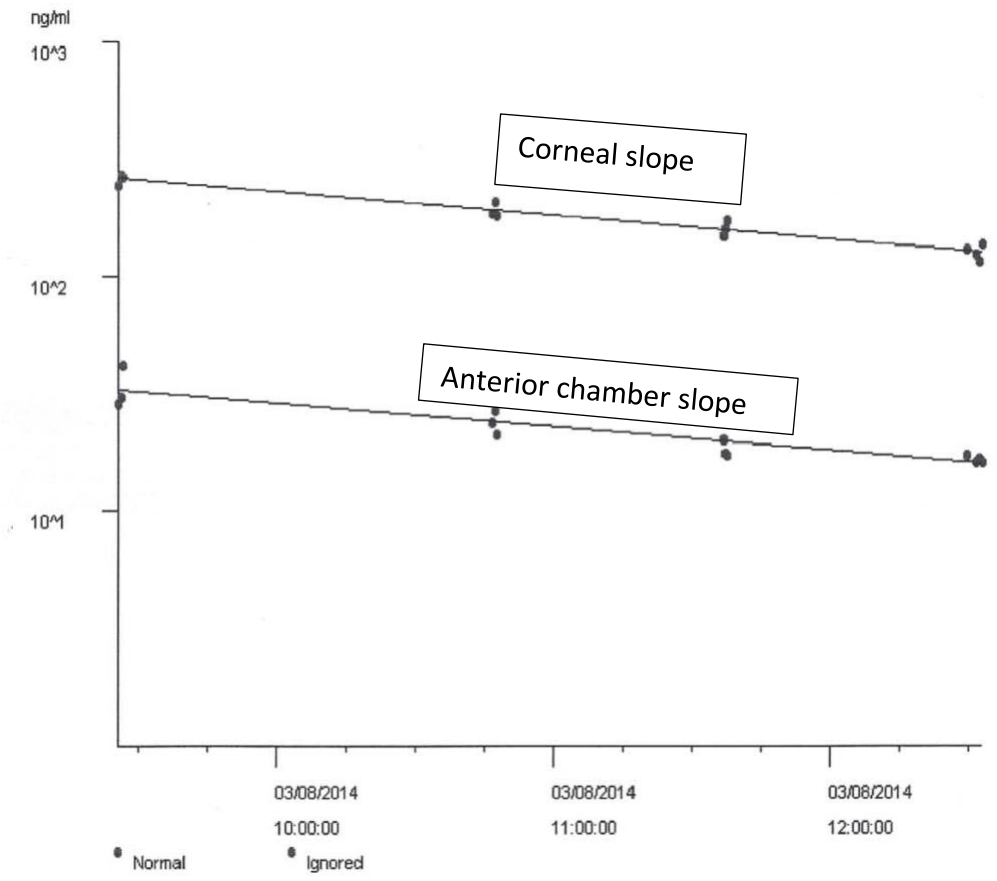
To calculate the aqueous humour flow rate , the Fluorotron software uses default variables that can be changed by the operator as necessary (Figure 2-3):

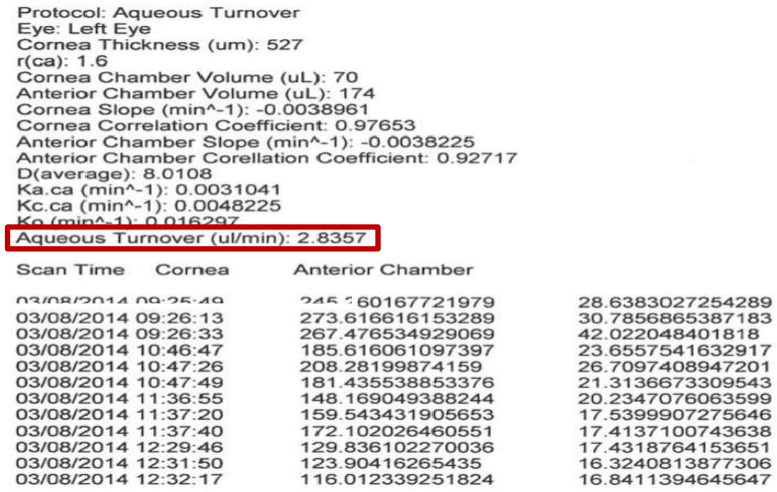
- Corneal volume default value, 70µL
- Anterior chamber volume default value, 174 µL
- Central corneal thickness (CCT) of each patient, in µm (I have used each patient's central corneal thickness instead of using the 500 µm default value to correct for the depth of the focal diamond).

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

The aqueous turnover analysis results are:

- Corneal slope
- Correlation coefficient: shows how closely the linear regression lines fit the cornea/anterior chamber points using the least squares analysis.
- D avg: Ratio of the midpoints of the two best-fit lines on the graph
- Ka.ca: diffusion coefficient referred to the anterior chamber volume, from cornea to anterior chamber
- Ko: diffusion coefficient due to outflow from the anterior chamber
- Aqueous turnover: Volume of aqueous passing through the anterior chamber per seconds.




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	245.60167721979	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 2-3 Fluorotron output data. The upper line shows the corneal concentration of fluorescein and the lower line depicts the anterior chamber fluorescein concentration. The software allows the operator to ignore unreliable measurements and labels them as "ignored". In this diagram all points have been considered as "normal"

Homogenous distribution of fluorescein in the cornea and anterior chamber is crucial in fluorophotometry (Brubaker and McLaren, 1985). To improve the uniformity of distribution of fluorescein in the anterior chamber, patients should be instructed to move their eyes to different directions before measurements. In terms of corneal distribution uniformity, participants should be advised to administer fluorescein in the middle of night (McLaren, 2009). This allows the corneal stroma to become saturated with fluorescein and create an equilibrium between stromal and aqueous fluorescein concentration.

When the fluorescein is applied topically, the aqueous humour flow rate is measured at 2.4±0.6 µl/min in healthy White Caucasian individuals (McLaren, 2009). There is no marked difference between men and women in terms of their aqueous humour flow rate. The aqueous humour flow rate is not significantly different in African/Caribbean and White Caucasian individuals (2.36 vs

2.26-ml/min) (Beltran-Agullo et al., 2011). The aqueous humour flow rate degrades 4% per each decade of life. There are different theories explaining this phenomenon including decline in number of ciliary epithelial cells, aging changes in the ciliary body structure and neuronal/hormonal alteration with age (Brubaker, 1991). Although, from 20 to 80 years of age the rate of aqueous humour flow rate reduces by 25%, the anterior chamber depth in phakic eyes is reduced by 40% (due to lens enlargement) and consequently the aqueous turnover is increased by 20% over a life time (McLaren, 2009; Toris et al., 1999). The aqueous flow rate can vary in some situations during day and wake time except sleep (and supine position), in which the flow rate is almost halved compared to wakefulness (1.3±0.4 µl/min) (Brubaker, 1982; Brubaker and McLaren, 1985; R. F. Brubaker, 2004; Larsson et al., 1995; Brubaker, 1991). In recumbent position venous return is increased and consequently the episcleral-venous pressure is increased. Therefore, in order to maintain the steady state of normal IOP in the eye, the aqueous flow rate is reduced. This suggests a circadian rhythm for the aqueous humour flow rate. The reason for this change is postulated to be related to several factors including reduced circulating catecholamines during sleep (sympathetic system is a drive for production of aqueous humour during wakefulness) and increased episcleral venous pressure. Nonetheless, this is just a simplification of a very complex system. The aqueous humour flow rate is highest in the morning (approximately 3.0 µl/min) while it is at its lowest at night-time during sleep when it is halved.

Water drinking test have shown to reduce aqueous flow rate for a very short period of time after the test, before it is restoring to pre-test values (Diestelhorst and Krieglstein, 1994; Arora et al., 2013).

To be able to reliably utilise fluorophotometry technique, few assumptions must be met (Brubaker and McLaren, 1985). They are listed as following:

- The time between the application of fluorescein and its measurement is long enough to allow saturation of the corneal stroma to take place and create constant ratio between cornea and anterior chamber. Additionally, the ratio of corneal stromal and cameral fluorescence is constant.
- The aqueous humour flow rate and the fluorescein penetration through corneal stroma is in a steady state.
- The stromal volume is 70 µl (this is an assumed value but could be measured with Pentacam). I have used the assumed value of 70 µl in my projects.
- The fluorescein diffusional loss is 0.25 µl/min (intact blood-aqueous barrier and uninflamed eye)(McLaren, 2009; Brubaker, 1982, 1991). In the presence of inflammation, the aqueous-blood barrier is broken which allows proteins (flare) to leak into the anterior chamber. This will subsequently increase scattered light which can then diminish the measured fluorescence. Additionally, fluorescein can bind to proteins and its fluorescence is quenched and decreased which in turn has an impact on the fluorescence signal (personal communication with

Dr-Jay-McLaren). Another condition which can influence the diffusional loss is rubeosis iridies.

- The iris-lens diaphragm is intact (phakic eye) and there is no artificial connection between the anterior and posterior chamber (i.e. peripheral iridotomy or iridectomy). Consequently, the presence of intraocular inflammation, intraocular surgeries with a breach of the iris-lens diaphragm such as cataract surgery, aphakia and widely dilated pupil would render this technique obsolete.
- At least a one-hour time interval between each measurement is required. In addition, to improve the accuracy, the measurements should be repeated several times and the average measurement should be taken into account.
- The amount of measured fluorescence of the anterior chamber is 99%. This is from the anterior chamber - and 1% stromal fluorescence. It can also be said that any condition which compromises the corneal clarity may make the fluorophotometry worthless.
- The amount of fluorescein in the cornea should be ≥200 ng/ml to reduce the corneal autofluorescence and stromal binding of fluorescein.
- The focal diamond of the fluorophotometer should be aimed on the cornea. The intersection of the illuminating beam and pick-up optical path defines a small area inside the anterior chamber called the “focal

diamond". This is the area where the fluorescence in the cornea and anterior chamber is detected by photodetectors.

- Corneal integrity and pathology can affect diffusion of fluorescein; therefore, it is crucial that the cornea is intact.

There is a sequence of events that leads to the production of aqueous humour fluid. The blood flows through the uveal tissue and circulates into the ciliary bodies. The ultrafiltration of plasma penetrates through the fenestrations of the blood vessels. The ciliary body's epithelium actively produces this filtered aqueous fluid. The blood flow rate (It is estimated to be 115 µl/min) to the ciliary body has been calculated indirectly by using the ascorbic acid level in the anterior chamber. The concentration of ascorbic acid in the aqueous is 20-fold higher than plasma in humans. The ascorbic acid is not catalysed in humans due to the lack of enzyme required to break down ascorbic. Therefore, if the ratio of aqueous-plasma of ascorbic acid and the rate of aqueous humour formation rate is known, then one can calculate the minimal rate of blood flow to ciliary processes (Linner, 1951). If we apply the size of human pars plicata to animal studies of blood circulation to the ciliary bodies, the blood flow is measured at 154 µl/min. The rate of plasma filtration is about 2.7 µl/min (approximately 5% of plasma in the ciliary bodies). The main driving force for the aqueous formation is the oncotic pressure difference between blood vessels and the anterior chamber. This process is an active secretion. Aqueous humour

flow rate is at its peak in the morning due to adrenergic response and then it is reduced throughout the day, causing an IOP changes between 3-6 mmHg in normal eyes (Brubaker, 1991). This has been discussed earlier under section about changes of aqueous flow rate during sleep.

The role of the aqueous humour is to nourish the intraocular structures; it brings antioxidants and maintains the firmness of the globe and intraocular pressure. The aqueous humour contains vitamin-C, nitric oxide (NOS), potassium and a trace amount of protein but that is close to zero. There is a fine balance between aqueous flow rate and outflow. Any disequilibrium would be disastrous to the ocular health. Too low outflow facility would lead to glaucoma. Too low of aqueous humour flow rate or too high of its drainage would cause hypotony. Both situations can ultimately lead to blindness.

Trabecular meshwork outflow

The beam-like configuration of the trabecular meshwork (TM) structure comprises of trabecular meshwork cells surrounded by connective tissue sheets and beams. This structure connects the Schwalbe's line to scleral spur and ciliary muscles and encircles the entire circumference of the anterior chamber angle recess (Carreon et al., 2017). Histologically, it can be split into three major parts:

- The uveal meshwork
- The corneoscleral meshwork
- The juxtacanalicular (JCT) meshwork

Direct micro-cannulation data have shown that the majority (90%) of resistance to outflow is between the anterior chamber and Schlemm's canal. The remainder of resistance to flow is located between the canal and episcleral veins (Bill, 1993). Investigators have utilised electron microscopy to calculate the number of pores in the inner wall of the Schlemm' canal. They have shown that there are approximately 20,000 pores in humans' trabecular meshwork. The largest pores are measured 3μm. The trabecular meshwork cells have phagocytic ability which keep the TM free of debris (Johnson et al., 1989). Furthermore, TM cells play an active role in modulating the inflammation (Shifera et al., 2010) which has been harnessed in some glaucoma treatment modalities such as laser trabeculoplasty (Latina et al., 1998). Another function of the TM cells is to generate some resistance to the outflow. This is mediated

by fibroblastic and smooth muscle like function of the TM cells (Keller et al., 2009). This feature enables TM cells to be contractile and with its counter-tension, the ciliary muscle contracts to prevent the collapse of the trabecular-meshwork (Stamer and Clark, 2017). Mechanical stress from eye rubbing, blinking, eye movements, lack of blood supply and continuous exposure to free radicals from ultraviolet (UV) exposure, debris and waste within aqueous humour, pose a special challenge to TM cells. Therefore, several adaptive measures have been created within TM cells such as oxidative reducing materials (Russell and Johnson, 1996), special cytoskeleton (Baetz et al., 2009) and phagocytic ability (Sherwood and Richardson, 1988). Depending on the location of the TM cells their behaviour is more like the endothelium in the uveal/corneoscleral region and more fibroblast like in the juxtacanalicular region. Glaucoma (Grant, 1951; Stamer and Clark, 2017) and the ageing process– both reduce the trabecular outflow facility by increasing the outflow resistance (Toris et al., 1999).

Another aspect of the trabecular meshwork which might be important in pathophysiology of raised IOP is trabecular meshwork stiffness. Last and associates (Last et al., 2011) assessed the compressive stiffness of the trabecular meshwork in glaucomatous eyes post-mortem. They showed that the stiffness of TM was much higher in glaucomatous eyes compared to the healthy eyes. Some observations in vivo and in vitro in human eyes showed that the extracellular matrix such as glycosaminoglycans (GAG) and proteoglycans are found

abundantly on the surface of the TM cells in the juxtacanalicular region. In perfusion studies using enzymes to degrade the extracellular matrix, increased trabecular outflow facility (Acott and Kelley, 2008). Additionally, it has been identified that in older eyes and in particular in glaucomatous eyes, there is a higher segmental flow (Buller and Johnson, 1994) and protrusion of extracellular matrix into the collector channels (Freddo and Gong, 2009).

Over the past few decades, there have been several attempts to measure the aqueous humour parameters. The first tonometer was described by Makaklof in 1885. Schiotz, Bock, Kronfeld, and Stough, and others all of whom observed that the ocular tension fell more slowly in glaucomatous eyes than in normal-eyes under the influence of the pressure after a tonometer was applied repeatedly at short intervals or after prolonged application (Scheie et al., 1956).

The major milestone, however, was the introduction of tonography by Grant in-1950 (Grant, 1950). He published the result of a large study containing 1000-tonography tracings in 1951 (Grant, 1951).

Nowadays, there are indirect and direct methods of measuring trabecular outflow facility. With regards to indirect methods of measuring trabecular outflow facility, two main techniques have been developed. The conventional method of tonography is the weighted tonometer such as Schiøtz tonometer (Grant, 1950) or pneumatonometer (Langham et al., 1968). In these approaches, the extra weight of the tonometer causes a rise in IOP that disturbs the steady-state within aqueous humour dynamics akin to an exercise stress test.

The rate of recovery of IOP to its usual pressure is measured during 4-minute period while the tonometer remains on the eye (Brubaker, 2004). Later, Hayashi and colleagues in 1989 (Hayashi et al., 1989) introduced a method of measuring the trabecular-outflow facility whereby the aqueous flow was suppressed pharmacologically, and then the rate of aqueous flow rate reduction was measured afterwards. The difference between the pre- and post-medication of aqueous flow rate was calculated. This was then used as a proxy for trabecular outflow facility.

Grant was the first to suggest Schiøtz tonography to measure the trabecular outflow facility. He spent many years on assessing the tonographic (trabecular) outflow facility of cadaveric eyes. He managed to refine his "weighted tonometry-technique" and finally, published his seminal paper in 1950 (Grant, 1950). He measured the trabecular outflow facility via direct methods and compared them with the Schiøtz tonography (Grant, 1951). The result was comparable. The electronic Schiøtz tonography machine consists of a probe (houses a plunger) that indents the cornea and exerts force on the cornea. It artificially increases IOP. The tonography is a technique of measuring hydraulic resistance and postulates an indirect measure of trabecular aqueous outflow. This test is usually performed for 4-minutes in supine positions (Alaghband et al., 2018; Beltran-Agullo et al., 2011; Brubaker, 1975). As the time passes the plunger indents the cornea and the displacements of the plunger within the

probe creates an electrical current which in turn is transmitted to the galvanometer. Subsequently it moves the pen connected to the galvanometer to produce a tracing on the moving paper. Grant called this value “The coefficient of aqueous outflow facility” (C).

The unit of tonographic outflow facility is µl/min/mmHg (microlitre of aqueous per millimetre of intraocular pressure per minute). This is essentially a quantitative illustration of how easily the fluid leaves the eye (the facility of outflow).

In my projects in this thesis, I measured tonographic outflow facility (*C*) by a constant weight tonography (5.5, 7.5 or 10 g) using a modified digital Schiøtz-tonographer (designed by the Department of Bioengineering, Imperial College, London, UK) (Alaghband et al., 2019) at 10 – 11 AM (Figure 2-4).

Figure 2-4 Schiøtz tonography machine

This device used an original Schiøtz tonographer footplate from a commercially available unit (model 720, Berkeley Bioengineering Inc., San Leandro, CA, USA) attached to a 3D printed shell that was designed such that the weight conformed to the specifications set out by the Committee on Standardization of Tonometers (Friedenwald, 1954)(Figure 2-5).

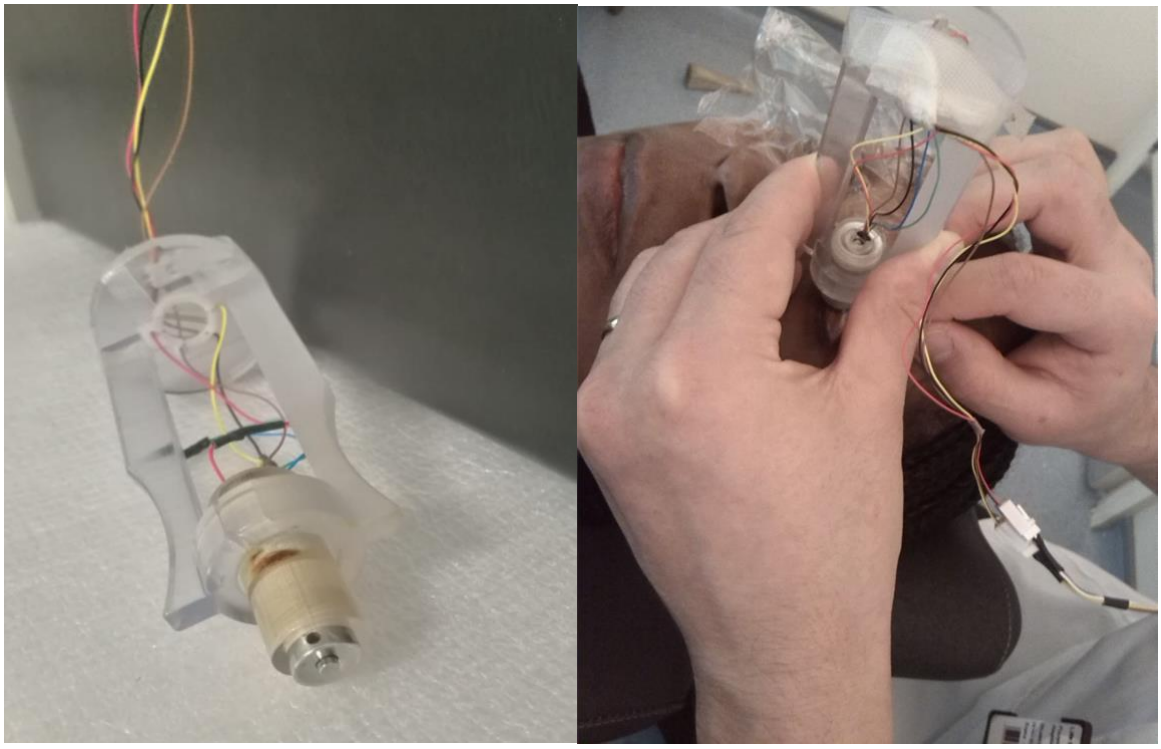


Figure 2-5 The 3D printed shell for tonography machine

Displacement of the weighted plunger was measured using a linear variable differential transformer (LVDT) (MHR series, TE Connectivity, Schaffhausen, CH, USA) driven by a signal conditioner (AD698, Analog Devices, Norwood, MA, USA) and captured digitally by a data acquisition system (USB-6009, National Instruments, Austen, TX, USA). Validation studies confirmed that the LVDT voltage output was linear with respect to the Schiøtz scale reading (

Figure 2-6), where each scale reading is equivalent to 0.05 mm of plunger displacement (Friedenwald, 1954). Using a micrometre, the plunger of the-LVDT was moved in increments of 50 µm, equivalent to 1 Schiøtz scale

reading, over the range of -1 to 20 scale readings, whilst measuring the LVDT voltage output (
Figure 2-6). Each position was measured 2 or 3 times, with all data shown. The voltage-displacement relationship was linear over the full range (R^2 = 0.9997), allowing the measured LVDT voltage to be converted into a Schiøtz scale readings. A scale reading of -1 was defined as the reading when the footplate was placed on a rigid spherical surface with a 15 mm radius of curvature (Friedenwald, 1954). This is an original work which is already published as part of the aqueous humor dynamics in uveitic eyes project (Alaghband et al., 2019).

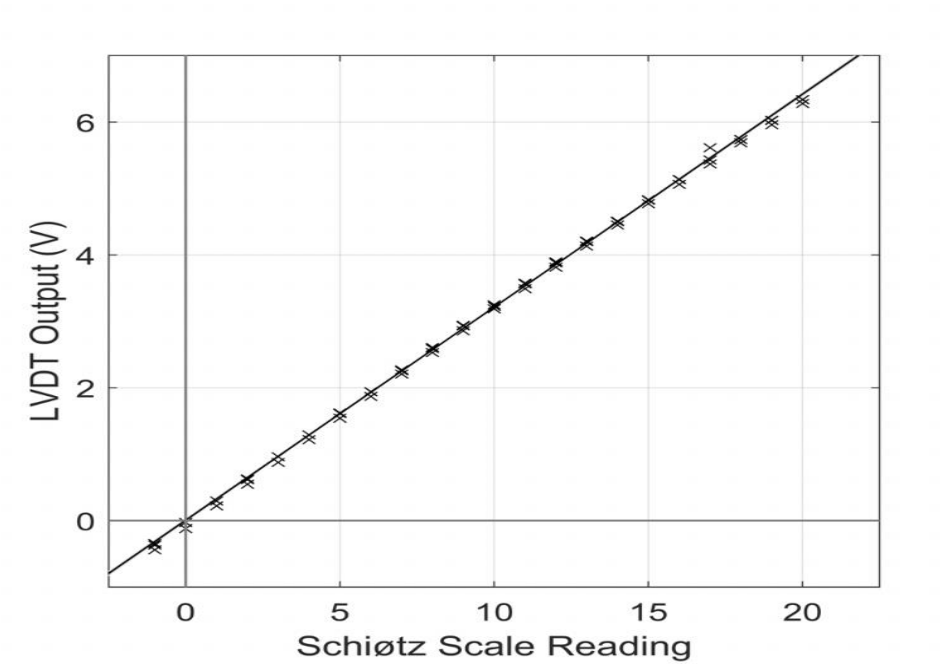


Figure 2-6. Shows the correlation between digital and electronic Schiotz tonography. The grey cross represents the original Schiotz readings and the black cross shows the modified digital Schiotz tracings (R^2 = 0.9997).

,mmHg

Facility was estimated using Grant's equation (Equation 2) (Grant, 1950).

Equation 2 Shows the Grant's original equation

$$C = \frac{V_{c,t} - V_{c,0} + \frac{1}{K}\left(log\ P_{t,0} - logP_{t,t}\right)}{\left(\frac{P_{t,0} + P_{t,t}}{2} - P_0 - \Delta P_v\right) t}$$

Where $V_{c,t}$ and $V_{c,0}$ are the aqueous volumes displaced at time t and at the start of tonography ($t = 0$). $P_{t,t}$ and $P_{t,0}$ are values of IOP at time t and at the start of tonography. P_0 is the IOP immediately prior to the start of the tonography. ΔP_v is the change in episcleral venous pressure (EVP), assumed to be 1.25 mmHg (Leith, 1963). It is thought that the pressure applied on the eye during tonography can increase intra-orbital pressure and subsequently causing congestion of orbital veins and raised EVP. K is the coefficient of ocular rigidity, assumed to be 0.0215/µl^{-1} (Friedenwald, 1948). The coefficient of ocular rigidity can be calculated using $k = \frac{LogP1 - Log\ p2}{V1 - V2}$, where V_1 and V_0 are the volumes indented in the eye by the tonometer when the pressure was P_1 and P_0, respectively. $V_{c,t}$, $V_{c,0}$, $P_{t,t}$, $P_{t,0}$ and P_0 were determined based on the value of the Schiøtz scale reading and tables provided by Moses and Becker (Moses and Becker, 1958). By minimising the root mean square error between the tonographic tracing and Equation 1 the optimal value of C was determined numerically. The black trace shows the captured signal from the LVDT, converted into Schiøtz scale readings (see Figure 2-6). The red line shows the predicted scale reading based on fitting **Error! Reference source not found.** to

the black tracing for the optimal value of *C* (in this case 0.31 µl/min/mmHg). Tonography was performed with a 5.5-gram weight placed on the right eye of a healthy volunteer (IOP = 16-mmHg) (Figure 2-7). The high frequency oscillations (~1 Hz) observed in the black tracing reflects the ocular pulse.

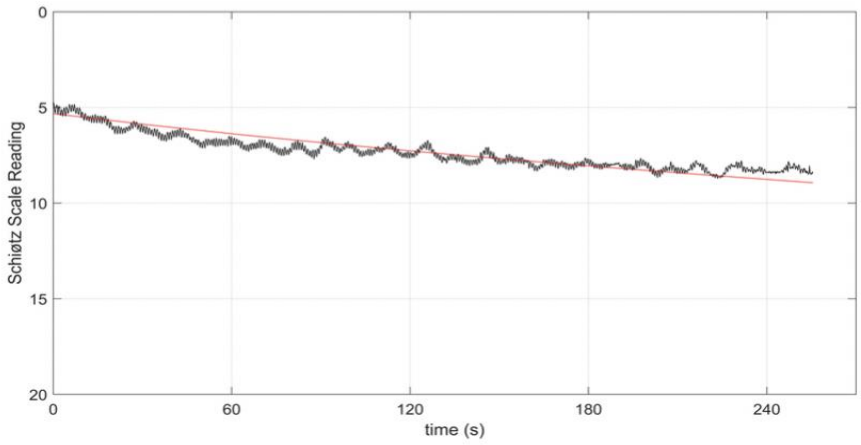


Figure 2-7. A sample tracing of the digital Shiøtz tonography

The recording Schiøtz tonometer I used in my studies had a concave tip with an external diameter of 10 mm. The total weight of the tip as applied to the cornea is 16.4 g (Figure 2-8).

Figure 2-8 Close-up photo of the Schiotz tonography probe

Later Langham (Langham et al., 1968) suggested pneumatonography as a means to measure trabecular outflow facility. This device too has a probe which is supported by pneumatic force to indent the cornea (Figure 2-9).

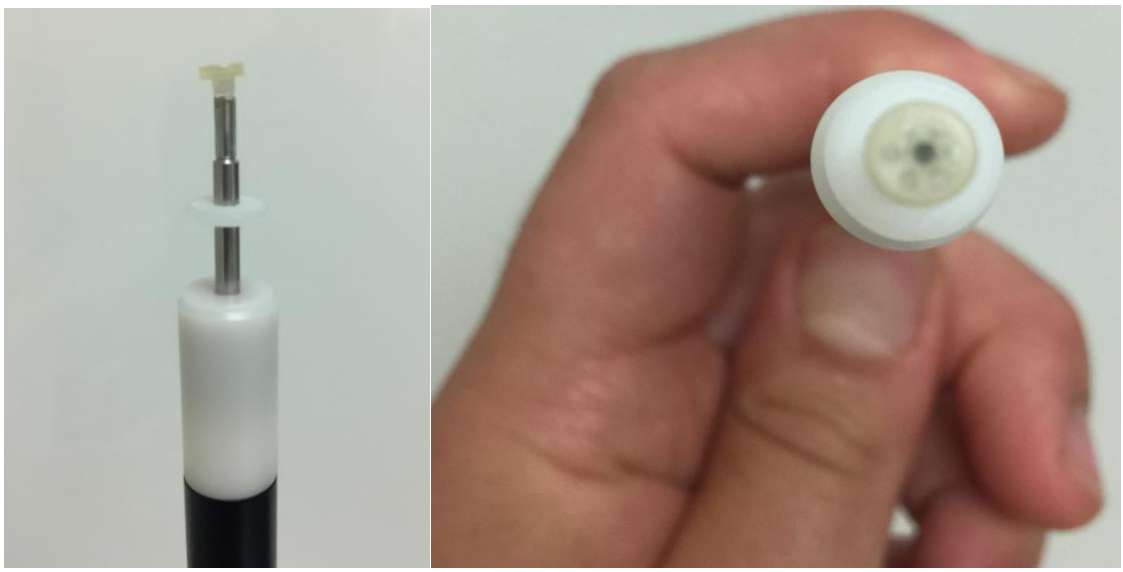


Figure 2-9 Close-up photo of pneumatonography probe

The total weight of the Schiøtz and pneumatonometer probes are similar (16.4 g and 14.7 g respectively), the difference in tip sizes; However, the concave base of the probe in Schiøtz is 10 mm vs. 5.3-mm in radius with a flat tip of pneumatonometer. This results in a 4-fold larger surface area for the Schiøtz tip; therefore, the smaller pneumatonometer tip can lead to a greater application of pressure (Lim et al., 2008). This could cause physical changes to the aqueous humour outflow system. Also, there is a larger intraobserver and inter-subject variability (Lim et al., 2008; Feghali et al., 1986). In a laboratory based study, Moses (Moses, 1967) commented that pneumatonography is unsuitable for the assessment of outflow facility because it underestimates the magnitude of changes in IOP, and the additional 10-g weight required for tonography causes

the scale reading to underestimate IOP readings during tonography. This may well be due to the greater mobility of the pneumatonometer probe on the corneal surface and the greater transmission of subject's head movements and the operator's hand tremor to the device. There is a guide ring around Schiøtz probe which dampens the transmission of any unwanted movement to the transducer. The debate over reliability of these two methods is eloquently discussed in Kazemi et al. paper (Kazemi et al., 2017). They compared two methods in 56 eyes of 28 participants. They found that there is a tendency for higher tonographic outflow facility readings by using Schiøtz tonography as opposed to pneumatonography. However, they calculated the scleral rigidity (Moses and Becker, 1958) for each participant and once they adjusted it for the tonographic outflow facility readings, both techniques were equally reliable; however, they suggested that the use of each method is not interchangeable. Both devices, Schiøtz tonography and pneumatonography, measure the decay of the acutely elevated IOP and assume aqueous flow rate changes are minimal during this time (Garner, 1965). The assumption is that the changes in IOP is only due to increased trabecular outflow facility during the tonography whilst aqueous flow rate (as a proxy of aqueous production) remains unchanged. The Schiøtz tonography has a standard weight of 5.5g for IOP below 20 mmHg, then 7.5g is used for 20-30 mmHg and finally, 10g for IOP >30 mmHg (Garner, 1965). The comparison for different trabecular outflow facility measurement techniques are shown in *Table 2-1*.

	Schiøtz tonography	**Fluorophotometry**	**Aqueous suppression technique**	**Pneumatonography**
Pressure alteration during the test	Pressure increased twice the baseline	unchanged	Pressure is reduced (halved the baseline)	Pressure increased twice the baseline
Test duration	4 minutes	4 hours	3 hours	4-minutes, 2-minutes
Ocular tissue volume	Indentation of cornea and expansion and contraction of sclera	unchanged	Sclera may contract but this change is negligible	Indentation of cornea and expansion and contraction of sclera
Aqueous flow	May alter slightly (pseudo-facility of Barany is within the assumption)	Unchanged	The flow is reduced in second part of the test	May alter slightly (pseudo-facility of Barany is within the assumption)
Episcleral venous pressure	Assumed to rise 1.25-mmHg	unchanged	unchanged	Assumed to rise 1.25-mmHg
Equilibrium	Assumed throughout measurement	Assumed throughout measurement	Assumed throughout measurement	Assumed throughout measurement
Medication administered	Not applicable	Fluorescein	Aqueous suppressants i.e. acetazolamide or timolol	Not applicable
Assumptions about the medication	Not applicable	Does not change the aqueous parameters	Does not change the aqueous parameters	Not applicable
Facility of outflow	Assumed to be stable during measurement and not affected by IOP	Assumed to be stable during measurement and not affected by IOP	Assumed to be stable during measurement and not affected by IOP	Assumed to be stable during measurement and not affected by IOP

Table 2-1. Comparison between existing methods of measuring human facility of outflow

Several assumptions have to be met in order to have a reliable tonography measurement. Indentation tonography makes no compensation for the individual variations in ocular rigidity. The stiffer the eye, the greater force required to indent the cornea and displace the aqueous humour. Nonetheless, the scleral rigidity coefficiency is assumed to be an average of $0.0215\mu L^{-1}$ (Friedenwald, 1954). Moreover, this figure is normally derived from cadaveric eyes for Schiøtz-tonography and in pneumatonography from living eyes (this is one of the shortcomings of Schiøtz tonography). Another assumption is that cornea is a perfect sphere.

Other assumptions are listed below (Drance and Carr, 1960; Feghali et al., 1986).

- The tonography is accurately calibrated
- Corneal flexibility is negligible
- The amount of fluid displaced during measurement is accurately known
- Scleral elasticity is known
- Ocular blood and tissue volume remain unchanged
- Aqueous humour flow rate (as a proxy of aqueous humour production) remains constant and not affected by IOP
- Episcleral venous pressure remains unchanged during tonogrpahy
- Intraocular fluid change is solely due to indentation of tonography
- The duration of test is long enough for the rate of IOP to decay

- The eye is in the steady state
- Pseudofacility is negligible

At the present time, tonography is the best available method measuring the trabecular outflow facility. In an experiment, Becker and Constant (Becker and Constant, 1956), performed tonography in-vivo and in vitro with perfusion methods of measurement of the outflow facility. They used 28 rabbit eyes and four human eyes which were due to be enucleated. They found good correlation between direct and indirect methods. They concluded that tonography is a reliable and accurate technique of measuring the trabecular outflow facility.

It has been known for a long time that compressing or contusing one eye can affect the other eye's IOP consensually (ophthalmotonic-consensual reaction). Barany and Wirth (Barany and Wirth, 1954), noticed that performing paracentesis in one rabbit's eye, can reduce the aqueous flow rate of the fellow eye. Other investigators (Prijot and Stone, 1956) noted that when one eye was compressed, the tonogram of the fellow eye became flat. Prijot and Stone (Prijot and Stone, 1956), applied compression on one rabbit's eye and observed reduction of IOP and aqueous flow rate of the fellow eye. This phenomenon is called "ophthalmotonic consensual reaction". The term was first coined by Weekers in 1924 (Weekers, 1924). Cambiaggi and Spurgen (Cambiaggi and Spurgeon, 1959), studied tonographic changes in a group of 16-healthy volunteers eyes and 24 glaucomatous eyes. They performed electronic-tonography on two separate days. On day one, tonography was first

performed on the right eye of each case followed by 4-minute intervals and then tonography of the left eye. On the second day, the order of measurement was reversed i.e. left eye first and then right eye and there was 2-minute interval between the two measurements. They demonstrated that the difference between the two eyes were not statistically significant. Although, the mean IOP of the second eye after initial tonography was slightly lower, it was not statistically significant. This change has been ascribed to the "vascular reflex". In this theory, it is suggested that the neural inputs may affect the episcleral venous pressure in both eyes during tonography. However, there is no strong evidence for this phenomenon (Leith, 1963). Nevertheless, the mean tonographic outflow facility measurement did not alter in either eye by tonography of the fellow eye when there is sufficient interval between each measurement. However, Grant and English (Grant and English, 1963) have shown that if the fellow eye is covered by something such as cling film to reduce the rate of evaporation of the tear, the chance of desiccation of the fellow eye would be minimised. Therefore, it is thought that tonography may change the ocular hydrodynamics (including hysteresis) only transiently.

Spencer et al. (Spencer et al., 1955) performed repeated tonography in 14-normal eyes to assess the repeatability of the test. They found that the average variation was ±0.045-µl/min/mmHg (19%) between the same eye of each participant. They demonstrated no diurnal variation. Additionally, they repeated the measurements on several days at different times of the day.

The mean variation was ±0.053-µl/min/mmHg (25%). They noted that the variability of the measurement was less if the tonography was performed at the same time of the day rather than at different time of the successive days.

Tonography is subject to some errors. Potential sources of error can be categorised as instrument, eye, patient and operator related.

- ***Instrument:*** error in design and manufacturing of the instrument
- ***Eye:*** variations of the corneal curvature, biomechanics and ocular rigidity
- ***Patient:*** fluctuations in systemic blood pressure, cardiac arrhythmias, exophthalmos, changes in breathing rate, coughing, Valsalva manoeuvre, contractions of extraocular muscles, removing tight tie or collar
- ***Operator:*** distraction and inattention to the position of the probe on the eye, anxiety of the operator and hand tremor, mislabelling of tracings and not enough retraction of eyelids

The normal value for the tonographic outflow facility ranges between 0.2-0.4-µl/min/mmHg. The cut off point for an abnormal tracing to signify a compromise in outflow facility is <0.2-µl/min/mmHg (Lim et al., 2008; Beltran-Agullo et al., 2011).

Episcleral venous pressure

Friedrich Schlemms (Leber, 1895) discovered the Schlemm's canal and Karl Ascher (Ascher, 1949) described the aqueous veins later in the 20th century. He suggested that episcleral veins are connected to the Schlemm's canal. He observed that the aqueous humour flows through Schlemm's canal and enters collector channels and drains into episcleral veins. Mixture of blood vessels (arteries and veins) make up the episcleral vasculature. At present, it is not clearly known how the episcleral veins are regulated. Grant and many other investigators have suggested that the majority of resistance to the outflow of aqueous humour is located at the distal section of Schlemm's canal (Grant et al., 1958). One possible explanation of this resistance may well be due to the special structure of the collector channels and episcleral veins. Recent studies by Johnston et al. have shown that the flow within the collector channels are controlled by a series of hinges and valves which can create further resistance to the aqueous flow (Johnstone et al., 2016). Goldmann (Goldmann, 1951) described a method of measuring episcleral venous pressure (EVP) by applying a constant pressure over the conjunctiva and look for blanching of the vessels. Then Linner (Linner, 1958), used a known force over the eye through a transparent distensible membrane. In this method, collapse of blood vessels were the endpoint of the measurements. Others have used direct cannulation of the episcleral veins to measure EVP. Brubaker et al. (Brubaker, 1967) compared

the torsion balance method (similar to Goldmann's technique), pressure chamber method and direct cannulation method. They concluded that even though the results of the measurements from each method were comparable, the direct cannulation method showed the least variability. In addition, the direct cannulation is based on fewer assumptions. In another study, Lim et al. (Lim et al., 2008) utilised an episcleral venomanometer (pressure chamber method) to measure EVP. In this method the balloon membrane was placed on the eye, close to a large scleral vessel. Under direct visualisation, the dial on the instrument is turned to increase the pressure within the balloon to collapse the vein. The endpoint was that the blood vessel to collapse half of its original diameter (half-blanched). The measurement value varied widely between participants and among the fellow eyes of the same participants and also with the measurements of the same eye of the same participant (few-minutes apart). Interestingly, the Valsalva manoeuvre did not affect the readings. They concluded that the measurement from this instrument was very inconsistent. The suggestion is to use the assumed value of 10 mmHg for EVP measurements. Sit and associates (Sit et al., 2011) developed a computerised venomanometry to measure episcleral venous pressure in a non-invasive manner. It improves the accuracy of identifying the compression of veins endpoint objectively. This technique was based on the pressure chamber method too. The device consists of a camera that captures the images of the episcleral vessels, a computer-controlled motor drives the pressure adjustments and

pressure transducer. This method was utilised in five healthy subjects' eyes to measure EVP. On average, EVP was 6.3 mmHg in healthy individuals using Sit et al. technique (however, the range can vary due to inherent variability/inaccuracy of the technique). Their measurements were constant between subjects. It is assumed that the variabilities in the EPV measurement arise from the lack of unanimity in identifying clear endpoints of the vascular collapse. Additionally, no comparison has been made between the direct methods to validate the result of Sit et al. technique. Nevertheless, it is the most objective yet indirect method of measuring episcleral venous pressure. The average EVP in the upright position varies between 7.6 and 11.4 mmHg depending on different studies. Interestingly, in studies on Sturge-Weber syndrome (Phelps and Armaly, 1978) which is believed that EVP to be high, the episcleral venous pressure measurements varied significantly. This again reiterates the uncertainties in the endpoint determination of blood vessels collapse. Alpha adrenergics appear to reduce episcleral venous pressure (C. Toris et al., 1995). However, the evidence is controversial, and some other studies did not show any significant change in EVP (C. B. Toris et al., 1995). New generation of glaucoma medications including rho kinase inhibitors (ROCK) are shown to reduce the episcleral venous pressure (Wang et al., 2015; Kazemi et al., 2018). Although, the EVP measurement (whether direct or indirect) has a great variability, the subjectivity in direct methods are considerably smaller compared with indirect methods (Brubaker, 1967).

However, indirect methods are the only available techniques to measure episcleral venous pressure in humans at present. In this thesis I have used the assumed EPV of 10 mmHg for calculating the uveoscleral outflow.

Unconventional outflow

Leber (Leber, 1895) demonstrated that after the injection of a tracer into the anterior chamber, most of it goes through the trabecular meshwork but some of it was found to be in the supra-choroidal space. Other studies which followed on afterwards, showed the tracer in areas such as sclera, and choroid. Fine (Fine, 1964) concluded that there must be an unconventional pathway of aqueous outflow. It was not until 1965 that Bill (Bill, 1965) set the foundations of our understanding of unconventional outflow pathways. This was a turning point in the history of aqueous humour dynamics. Now having equipped with the new knowledge of unconventional pathways, the imbalance of the Goldmann equation could be accounted for. Bill (Bill, 1965) perfused anterior chamber of monkey eyes with labelled tracers and observed different areas that the tracer exited from the eye. He showed that a significant percentage of aqueous humour drainage bypassed the trabeculum (about 20%) and the remainder of the tracer was found in the choroid, sclera and ciliary bodies. From there, the fluid either was absorbed into the choroidal circulation or seeped through the sclera into the orbital tissues. Bill referred to this pathway as the 'unconventional' pathway to differentiate it from "conventional" trabecular outflow. Now this definition has expanded to include corneal, iris and retinal pathways too. Bill in 1977 (Bill, 1977) illustrated that the iris and corneal route are insignificant. If retina remained attached, the pumping mechanism of the

retinal pigment epithelium which is the driving force for the retinal flow of aqueous humour, is minute. Bill in his animal experiments showed that the flow of aqueous humour via unconventional pathway is not dependent on IOP and it accounts for 20% of the aqueous outflow. The aqueous enters the uveal tract and then through ciliary bodies and then gains access to supra-choroidal space. However, there is much debate about what is the fate of this part of aqueous humour from here. Some suggested that it will dissipate into the orbital tissues and absorbed. Others have proposed that it drains through the vortex veins (uveo--vortex outflow). More recently, some investigators have proposed that this fluid may drain into the ciliary lymphatics (uveo-lymphatic pathways). Unconventional outflow, regardless of which pathway it is passing through (uveo-scleral, uveo-vortex or uveo-lymphatic), it should cross the ciliary body tissue (Johnson et al., 2017). It is difficult to measure the rate of the unconventional outflow quantitatively. Additionally due to diffused flow pattern of the unconventional outflow, it is challenging to pursue its path accurately. Nonetheless, two methods have been described, either direct measurements by utilising labelled tracers or indirect methods. The former is the gold standard, but it is invasive and not ethical for humans (due to the risk of radiation). In indirect methods, unconventional flow is calculated by deduction from the difference between the aqueous humour flow rate and trabecular (tonographic) outflow facility using a modified version of the Goldmann equation. However, the result of this method is varied considerably and does not match the values of

unconventional outflow derived from the direct measurement techniques. Overall, the indirect method over-estimates the unconventional outflow. The main issue of using the indirect-method is, the calculation is subject to many assumptions. Furthermore, the Goldmann equation is based on the assumption that trabecular (tonographic) outflow and episcleral venous pressure are independent of IOP.

In the living eye, uveoscleral and uveo-vortex pathways both contribute to unconventional outflow. Therefore, using the term uveoscleral outflow (extra-trabecular pathway) would be confusing. Johnson et al. (Johnson et al., 2017) suggested the term non-trabecular outflow as a more representative of the description; however, some investigators resorted to unconventional outflow which is more acceptable. I have used uveoscleral outflow term in the rest of this thesis.

Table 2-2 shows comparison between conventional outflow and unconventional outflow.

	Trabecular outflow (conventional)	**Non-trabecular outflow (unconventional)**
Pressure sensitivity	Pressure dependent	Pressure-independent (between 4-35 mmHg)
Proportion of outflow	80%	20%
Influence of ciliary muscle contraction after application of pilocarpine	minimal	Controls most of flow (approximately 90%)
Difference between living and post-mortem tissue	same	Increased significantly after death (due to loss of ciliary muscles tone)
Flow rate	0.2-0.4 µl/min/mmHg	0.4–0.6 µl/min
Effect of aging	Aging reduces the outflow	Aging reduces the outflow
Glaucoma	Decreased flow	Decreased flow
Main area of resistance	Trabecular meshwork, Schlemm's canal and episcleral veins	Ciliary bodies

Table 2-2. Comparison between conventional and unconventional outflow pathways

In the modified Goldmann equation, the unconventional outflow (U) is regarded as a constant that can be altered according to changes in IOP. However, unconventional outflow pathway is pressure independent. Hence, including driving forces of unconventional pathways and the pseudo-facility (Moses et al., 1985), would enhance the aqueous humour dynamics model. Interestingly, when ciliary body is removed such as in cyclodialysis cleft, the unconventional

outflow resistance would be circumvented. The unconventional outflow would then increase significantly and becomes pressure dependent.

Aqueous humour dynamics measurement methods used in this thesis

Aqueous flow rate measurement

The night before (10 PM) the fluorophotometric scans, participants self-administer from 3 to 5 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 < 26 years, 5 drops; age 26–35 years, 4 drops; >35 years of age, 3 drops) (Brubaker et al., 2001). Fluorophotometry is performed in both eyes with a scanning ocular fluorophotometer (FM-2, Fluorotron Master ocular fluorophotometer; OcuMetrics, Mountain View, CA) from 9 AM to 12 noon. The aqueous flow rate is determined using dedicated software provided with the fluorophotometer. Triplicate scans are collected and repeated at 1-hour intervals for four measurements to determine the aqueous humour flow rate (Ff). Following each set of scans, IOP is measured using pneumatonometry (Model 30 Classic; Reichert Ophthalmic Instruments, Depew, NY); IOP is recorded as the arithmetic mean of a total of 12 measurements per eye (3 measurements every hour alternating between eyes).

Tonographic outflow facility measurement

Tonographic outflow facility (*C*) is measured by constant weight tonography (5.5. 7.5 or 10 g) using a modified digital Schiøtz tonographer (designed by the Department of Bioengineering, Imperial College, London, UK) at 10 – 11 AM.

It is assumed that tonographic outflow facility is the measured trabecular outflow facility. In this device I used an original Schiøtz tonographer footplate from a commercially available unit (model 720, Berkeley Bioengineering Inc., San Leandro, CA, USA) attached to a 3D printed shell that was designed such that the weight conformed to the specifications set out by the Committee on Standardization of Tonometers (Friedenwald, 1954). Displacement of the weighted plunger was measured using a linear variable differential transformer (LVDT; MHR series, TE Connectivity, Schaffhausen, CH, USA) driven by a signal conditioner (AD698, Analog Devices, Norwood, MA, USA) and captured digitally by a data acquisition system (USB-6009, National Instruments, Austen, TX, USA). Validation studies confirmed that the LVDT voltage output was linear with respect to the Schiøtz scale reading (Figure 4-2), where each scale reading is equivalent to 0.05 mm of plunger displacement (Friedenwald, 1954). Facility was estimated using Grant's equation (Equation 2) (Grant, 1950).

$$C = \frac{V_{c,t} - V_{c,0} + \frac{1}{K}\left(log\ P_{t,0} - logP_{t,t}\right)}{\left(\frac{P_{t,0} + P_{t,t}}{2} - P_0 - \Delta P_v\right) t} \qquad Equation\ 2$$

where $V_{c,t}$ and $V_{c,0}$ are the aqueous volumes displaced at time t and at the start of tonography ($t = 0$). $P_{t,t}$ and $P_{t,0}$ are values of IOP at time t and at the start of tonography. P_0 is the IOP immediately prior to the start of tonography. ΔP_v is the change in episcleral venous pressure, assumed to be 1.25 mmHg

(Friedenwald, 1948), and K is the coefficient of ocular rigidity, assumed to be 0.0215/µl^{-1} (Moses and Becker, 1958). $V_{c,t}, V_{c,0}$, $P_{t,t}$, $P_{t,0}$ and P_0 were determined based on the value of the Schiøtz scale reading and tables provided by Moses and Becker (Moses and Becker, 1958). By minimising the root mean square error between the tonographic tracing (

Figure 4-3) and Equation 1 , the optimal value of C was determined numerically.

Uveoscleral outflow measurement

At present clinical measurement of the uveoscleral outflow in humans is not possible (Johnson et al., 2017); hence this value is generally calculated from the Goldmann's equation. Sit and McLaren used a computerized venomanometry to measure episcleral venous pressure (EVP) (Sit and McLaren, 2011; Brubaker, 1967). They illustrated that EVP in normal subjects can vary between 6 and 10-mmHg. Therefore, I have used 10 mmHg for our calculations.

Uveoscleral outflow was calculated using Goldmann's equation (Equation 3) with an assumed episcleral venous pressure of 10 mmHg.

$$Ff = (Pi - Pe)C + Fu \qquad Equation\ 3$$

"*Ff*" is the rate of aqueous humour formation measured by fluorophotometry, "*C* "is the tonographic facility of outflow, "*Pi*" is the intraocular pressure, "*Pe*" is the episcleral venous pressure, and "*Fu*" is uveoscleral flow.

Therefore,

$$Fu = Ff - C(Pi - Pe)$$

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.

Chapter 3|The effect of phacoemulsification on outflow facility

This chapter is presented as a published paper.

Pouya Alaghband , Laura Beltran-Agulló, Elizabeth A Galvis, *et al* Effect of phacoemulsification on facility of outflow. *British Journal of Ophthalmology* 2018;**102:**1520-1526.

Introduction

Several studies explored the effects of phacoemulsification on intraocular pressure (IOP) changes at short (6 months), medium (36 months) and long term (60 months) (Merkur et al., 2001; Mathalone et al., 2005; Peräsalo, 1997; Shingleton et al., 2006; Mansberger et al., 2012). The reported IOP reduction varies between 1.5 and 9.0 mmHg (Slabaugh et al., 2014; Mansberger et al., 2012; Pfeiffer et al., 2015). Different mechanisms of action have been proposed for the IOP lowering effects following cataract surgery, including the mechanical influence of the lens removal (Slabaugh et al., 2014), increased uveoscleral outflow, and increased trabecular outflow (Dooley et al., 2010). However, there are no studies using electronic Schiøtz tonography assessing the effect of modern small incision phacoemulsification with intraocular lens implantation on tonographic outflow facility.

Lee and Trotter (Lee and Trotter, 1957) investigated the effect of extracapsular cataract extraction without intraocular lens implantation on the facility of outflow. They used electronic Schiøtz tonography in patients with cataract of whom seven cases had open or closed angle glaucoma and 11 cases of

pseudoexfoliation. They showed that facility of outflow decreased within first 3 weeks postoperatively but then returned to preoperative values within 4 months after the operation. However, in this study outflow facility changes were very variable. Additionally, they had included mixed cases of complicated surgeries such as vitreous loss and the follow-up was only 6 months.

Another study by Meyer and associates (Meyer et al., 1997), demonstrated that pneumatonographic outflow facility after phacoemulsification improved on the first day after surgery; however, outflow facility at 1 year (0.46±0.38-µl/min/mmHg) was not statistically different compared to the baseline of 0.39±0.23 µl/min/mmHg.

To date there have been no studies using electronic Schiøtz tonography (which is a method of outflow facility measurement with less inter-subject and inter-observer variability compared to pneumatonography (Lim et al., 2008; Grant, 1951; Feghali et al., 1986)) to determine the effect of modern small incision phacoemulsification cataract surgery on tonographic facility of outflow.

Materials and Methods

Ethics approval for this study was obtained from the St. Thomas' Hospital local research ethics committee. This research conformed to tenets of the Declaration of Helsinki. Patients with visually significant cataract with primary open angle glaucoma (POAG) or cataract with otherwise healthy optic disc and no diagnosis of glaucoma, who were due to undergo phacoemulsification with

intraocular lens implantation, were enrolled in this prospective study. The POAG cases did not have washout from their hypotensive medications prior to the study measurements. The recruitment took place between September 2009 and May 2011. POAG was defined as raised IOP on at least one occasion and abnormal visual field testing with corresponding optic disc changes (patients with normal tension glaucoma were not included in this study). Only one eye per patient was included in the final analysis (all measurements were performed on both eyes). When both eyes were eligible, only the first eye to be operated on was included in the analysis (the alternative method would have been to include both eyes and use between the two-eye correlation). Patients were provided with study information at the initial contact, and signed informed consent was sought before measurements and the surgery. Patients with any previous history of intraocular or keratorefractive surgery, normal tension glaucoma, any secondary glaucoma including traumatic, neovascular, uveitic, pseudoexfoliative and pigment dispersion syndrome were excluded.

Contralateral eyes of the study patients, which did not undergo any intraocular surgery during the follow up period, with available tonographic outflow facility (TOF) and IOP data at all time points post operatively, were used for the comparison purposes.

Measurements

All patients underwent a comprehensive ophthalmic examination before the operation; including visual acuity measurement (LogMAR), slit lamp biomicroscopy, gonioscopy (using 2-mirror magnaview goniolens, Ocular Inc., WA, USA), intraocular pressure (IOP) measurement using Goldmann's applanation tonometer, anterior chamber depth (ACD) and axial length (AXL) measurement using IOL Master (Carl Zeiss Meditec Inc., Dublin, CA), central corneal thickness (CCT; Pachmate DGH 55, DGH Technology, Inc., Exton, PA), static automated visual field (Humphrey automated white-on white, 24-2 SITA-standard; Carl Zeiss Meditec), and dilated ophthalmoscopic examination.

Tonographic outflow facility (TOF = "*C"*) was measured with an electronic Schiøtz tonographer (model 720; Berkeley Bioengineering Inc., San Leandro, CA) on the day of the surgery (between 9 and 11 am) prior to the operation and then repeated at 3, 6 and 12 months postoperatively. The facility of outflow was measured from the rate of the decay of IOP in the supine position during application of a recording Schiøtz tonometer probe on the cornea, over a period of 4 minutes with a standard 5.5-g weight (Grant, 1951). Nine readings at 30-second intervals were manually entered the McLaren tonography computer program (Lim et al., 2008). The program fits a second-degree polynomial by the method of least squares to nine data points and determines by extrapolation, the best-fit values at time 0 and 4 minutes. The values at 0 and 4 minutes are then used to calculate "*C*" based on standard nomograms (Grant, 1951, 1950).

Surgical procedure

Cataract surgery was performed by an experienced surgeon (KSL) under local or general anaesthesia. A clear corneal incision (2.8 mm) was made and followed by a paracentesis and the injection of viscoelastic. Then capsulorrhexis and hydro-dissection was performed. The lens was removed by phacoemulsification of the lens nucleus and aspiration of the cortical lens matter. After further injection of the viscoelastic, an acrylic injectable intraocular lens (AcrySof® SA60AT, Alcon, Texas, USA) of the appropriate power was inserted into the capsular bag. The viscoelastic was washed out and an intracameral antibiotic injection was given at the end. Patients received Maxitrol® (Neomycin sulphate 3500 IU/ml, Polymyxin B sulphate 6000 IU/ml and 1 mg Dexamethasone, Alcon lab., UK) four times daily for two weeks and then twice daily for another two weeks postoperatively. All patients were off steroid treatment after this period.

Data Collection and Outcome Measures

Data including age, gender, race, IOP, TOF, CCT, axial length, anterior chamber depth, gonioscopy, vertical cup: disc ratio, and mean deviation in visual field testing were recorded.

Primary outcome measures were TOF (*C*) and IOP at 3, 6- and 12-months' post cataract extraction.

Sample size calculation

This study had a 90% chance of finding a 5% difference in IOP, and 7.5% difference in outflow facility if these differences existed (n=30 subjects, alpha-0.05, and beta 0.10). This is assuming that the baseline IOP and TOF is similar in POAG and cataract only groups. However, the current study was underpowered considering that only 27 patients were included in the final analysis.

Statistical analysis

Student's paired *t-test* and one-way analysis of variance for repeated measures (ANOVA) were used to compare continuous variables among groups. The 95% confidence intervals (CI) for the mean difference between pairs for each outcome measure were calculated. Linear regression analysis was used to determine the correlation between IOP, facility of outflow, and difference from baseline. $P < 0.05$ was considered to be significant (IBM SPSS. V23.0, IL, Chicago, USA).

Results

Forty-one patients were enrolled in the study. Eight patients withdrew after signing the consent, due to their time constraints and inability to attend all required post-operative visits. Three subjects had poor tonography tracings on at least one time point (10% rejection rate in aqueous humour dynamic measurement is similar to previous studies) (Lim et al., 2008). One case was

excluded due to an intraoperative complication (posterior capsular rupture and vitreous loss) and two further cases were omitted due to persistent post-op uveitis. In total, data from 27 patients with reliable tonographic outflow tracings at baseline and all subsequent study visits, were included in the final analysis. Only one eye from each patient was used for the analysis.

Sixteen individuals had cataract only, whilst 11 cases had an existing diagnosis of POAG. The average age was older in POAG group but the difference was not statistically significant (in cataract cases mean age was 67±11.2 years, while in POAG group it was 73±7.2 years, p=0.09). Other baseline characteristics of each group is shown in Table 3-3

*Table 3-3. Baseline characteristics. IOP: intraocular pressure, ACD: anterior chamber depth, AXL: axial length, CCT: central corneal thickness, BCVA: best corrected visual acuity, HVF: Humphrey visual field. * statistically significant, †Student paired t test (two sided), ‡ Mann Whitney U test*

	Cataract only (n=16)	**POAG and cataract (n=11)**	**P value**	**95% CI for difference**	**Effect estimate**	**Overall (n=27)**
Mean Age (years)±SD†, range	67±11.2 (43-82)	73±7.2 (59-83)	0.09	-14.8-1.1	-0.66	69±10.2 (43-83)
Gender (F:M) ‡	9:7	3:8	0.4	---	---	12:15
Mean BCVA±SD	0.3±0.4	0.3±0.2	0.7	-0.2-0.3	0.14	0.3±0.3
Ethnicity (Asian: African/Caribbean: White)	0:4:12	2:6:3	0.5	---	---	2:10: 15
Mean ACD (mm)†±SD, range	3.25±0.36 (2.9-3.8)	3.22±0.48 (2.85-4.1)	0.8	-0.3-0.3	0.07	3.23±0.40 (2.85-4.1)
Mean AXL (mm)†±SD, range	23.3±0.8 (22.8-24.5)	23.9±0.94 (22.5-25.7)	0.06	-1.3-0.05	-0.71	23.6±0.89 (22.5-25.7)
Mean CCT (µm)†±SD, range	535±27 (497-596)	543±27 (507-580)	0.5	-29.4-14.1	-0.28	538±26 (497 – 596)
Mean IOP (mmHg)±SD , range	15.7±2.8 (10-22)	16.3±4.8 (10-26)	0.7	-3.50 – 2.4	-0.16	15.9±3.66 (10-26)
Mean TOF (µl/min/mmHg)±SD, range	0.15±0.06 (0.06-0.29)	0.13±0.08 (0.04-0.29)	0.4	-0.03–0.08	0.33	0.14±0.06 (0.04– 0.29)
Median HVF (Mean deviation) ‡, range	2.5±0.6 (1.0-3.0)	-11.7±1.9 (-14.0-2.0)	<0.001*	----	---	-3.1±8.84 (-14 - 3)
Cup disc ratio‡	0.3 (0.2-0.7)	0.6 (0.6-0.9)	<0.001*	----	---	0.5 (0.2-0.9)
Mean Phaco power±SD, %, range	19.8±8.7 (10-31)	15.9±5.0 (10-27.9)	0.06	-2.1-9.9	0.52	18.2±7.5 (10 – 31)
Mean Phaco time±SD, minutes, range	2.25±4.12 (1.0-3.4)	1.54±0.54 (0.5-2.3)	0.16	-1.8-3.3	0.24	1.9±3.13 (0.5 – 3.4)

Baseline IOP was similar in each group (15.7±2.7mmHg in non-glaucomatous cases vs 16.3±4.8 mmHg in POAG with cataract group, p=0.7, 95% CI:-3.6–-2.4). Cases with POAG did not have washout from their hypotensive medications.

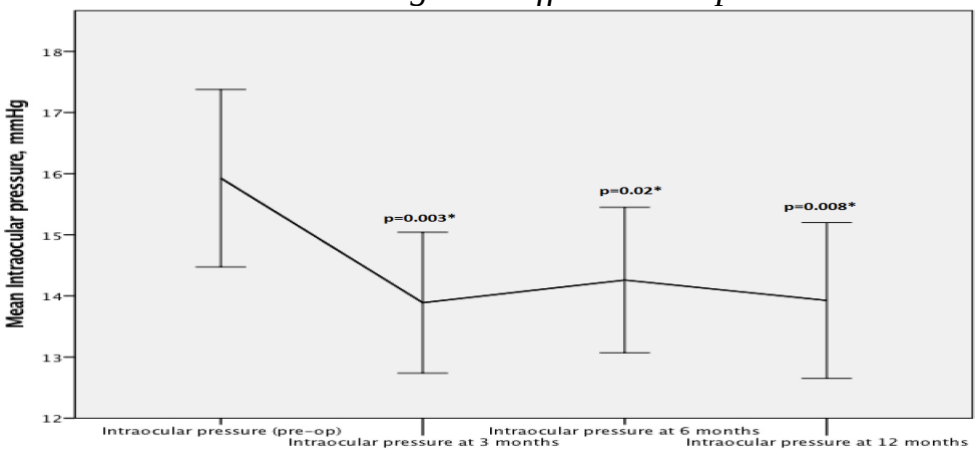
Overall, IOP reduced by 2.0±3.2 mmHg at 3 months (12% decrease), while at 6 and 12-month visits, it only reduced by 1.7±3.4 (10% decrease) and 2.0±3.6 mmHg (10% decrease) respectively (Table 3-4 **&** Figure 3-1). We used one--way-ANOVA for repeated measures to compare IOP between each visit. The IOP reduction at all post-op visits was statistically significant (3 months p=0.003, 6 months p= 0.04- and 12-months p= 0.02).

Table 3-4. This table illustrates IOP reduction at post op visits at 3, 6 and 12 months compared with baseline IOP. Additionally, percentage of IOP reduction is shown.

	IOP at baseline, mmHg	**IOP at 3m mmHg, % of reduction**	**IOP at 6m mmHg, % of reduction**	**IOP at 12m mmHg, % of reduction**
Cataract group (n=16)	15.7±2.8	13.4±2.7*, (13%)	14.4±2.6*, (7%)	13.7±2.9*, (12%)
POAG group (n=11)	16.3±4.8	14.5±3.2, (6%)	14.0±3.6, (9%)	14.2±3.7, (8%)
Overall (n=27)	15.9±3.7	13.9±2.9* (12%)	14.3±3.0*, (10%)	13.9±3.2*, (10%)

* statistically significant

Figure 3-1 Shows intraocular changes at different time points



TOF improved significantly at all post-op time points after cataract extraction compared to the baseline (

Figure 3-2 and

Table 3-5). However, TOF enhancement did not differ between each visit using one-way ANOVA for repeated measures (at 3 months p=0.7, at 6 months p=0.4 and at 12 months p=0.2). There was no statistically significant correlation between phaco power and TOF at any time point (at 3 months p=0.5, at 6 months p=0.4 and at 12 months p=0.7).

Figure 3-2 Shows the tonographic outflow facility changes at different time points postoperatively

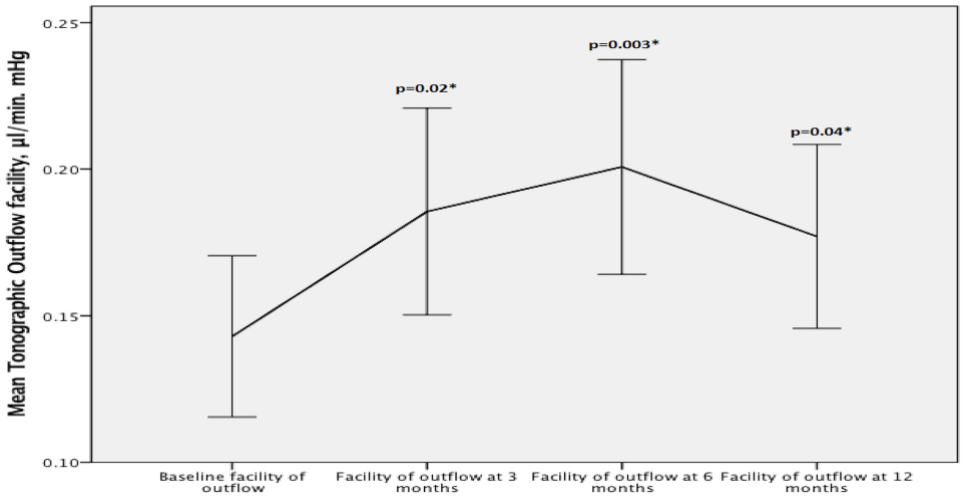


Table 3-5. This shows TOF enhancement post-surgery at each post op visit at 3, 6 and 12 months compared with baseline TOF. Additionally, the percentage of TOF enhancement is shown

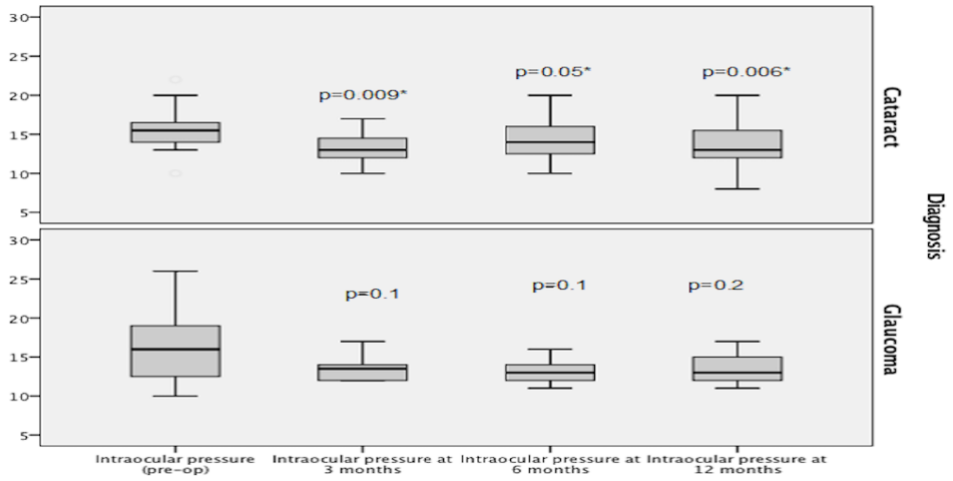
	TOF at baseline, µl/min. mmHg	**TOF at 3m (µl/min. mmHg), % of enhancement**	**TOF at 6m (µl/min. mmHg), % of enhancement**	**TOF at 12 (µl/min. mmHg), % of enhancement**
Cataract group (n=16)	0.15±0.06	0.20±0.09* (15%)	0.22±0.10* (17%)	0.18±0.07 (10%)
POAG group (n=11)	0.13±0.08	0.15±0.06 (14%)	0.17±0.07* (16%)	0.17±0.08 (15%)
Overall (n=27)	0.14±0.06	0.18±0.08* (16%)	0.20±0.09* (15%)	0.17±0.08* (10%)

* statistically significant

The average post-op IOP in cataract and POAG cases are shown in Table 3-4 and

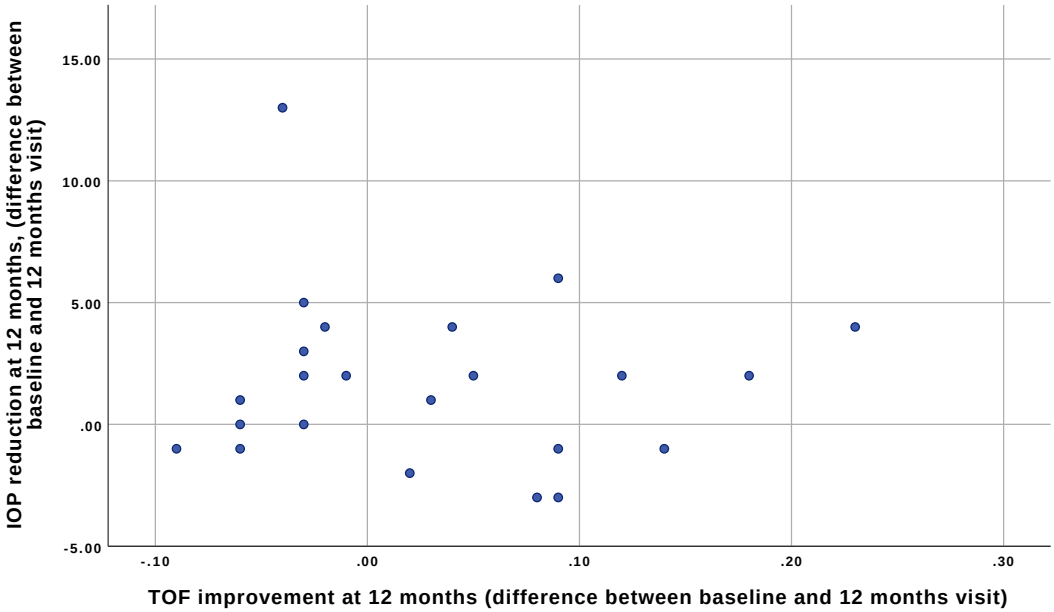
Figure 3-3. Mean IOP in cataract cases at 12 months' post-surgery was 13.7±3.0-mmHg; this was comparable to POAG cases in which IOP decreased to 14.2±3.7-mmHg (p=0.7, 95% CI: -3.0 – 2.2). Overall, baseline IOP was a moderate predictor of post-operative IOP reduction at all time points (p=0.004, r=0.53).

Figure 3-3. Intraocular pressure changes over 12 months' period compared between each visit. * statistically significant. (The vertical bars represent range; the boxes outer border shows the interquartile range and the thick middle line represents the mean) * statistically significant



Additionally, there was no statistically significant correlation between TOF changes and IOP alterations at 12 months (p=0.08, r^2=0.1) (Figure 3-4). Furthermore, there was no statistically significant correlation between AXL and ACD with IOP and TOF (p=0.9, r^2=0.002).

Figure 3-4. shows Spearman correlation between TOF changes and IOP alterations at 12 months. There is no linear correlation (p=0.08, r^2=0.1)



The number of glaucoma medication and treatment regime remained unchanged in the POAG group at baseline and at all post-operative visits. All patients were

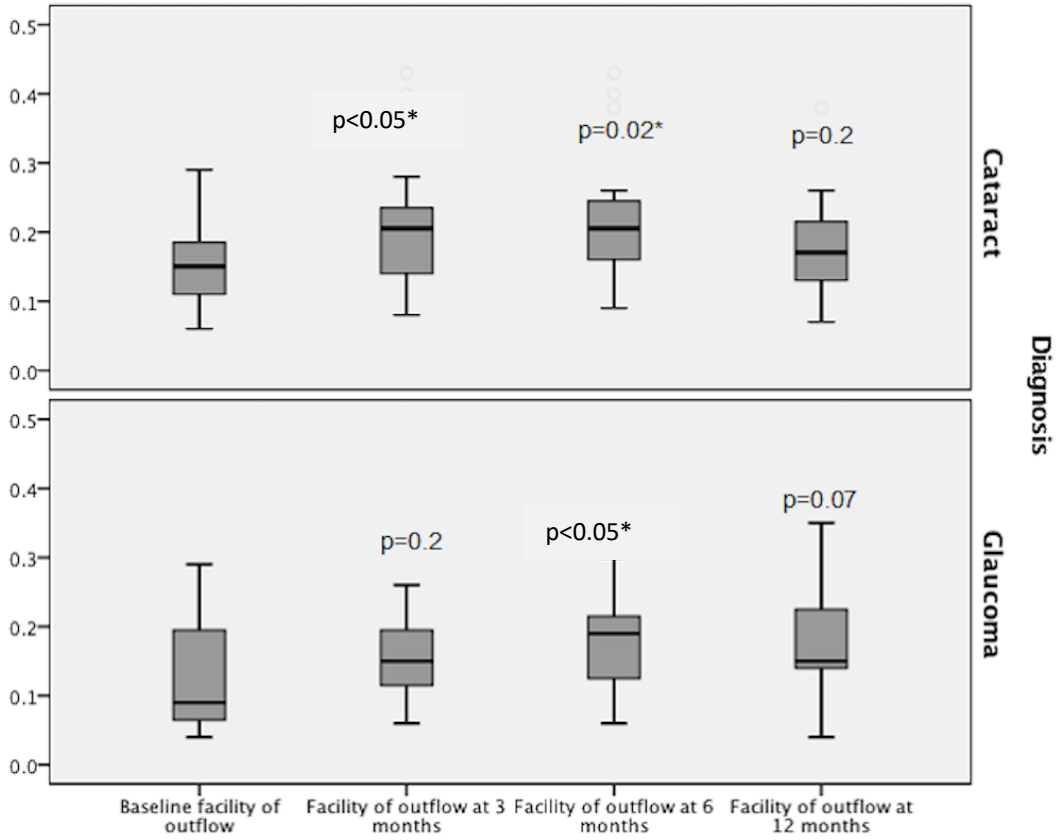
on prostaglandin analogues. Additionally, five patients were taking beta blockers, four patients carbonic anhydrase inhibitor and one patient was on alpha-2 agonist medications.

The IOP reduction was statistically significant at all post-op visits in cataract groups but not in the POAG group (

Figure 3-3 **and** Table 3-4).

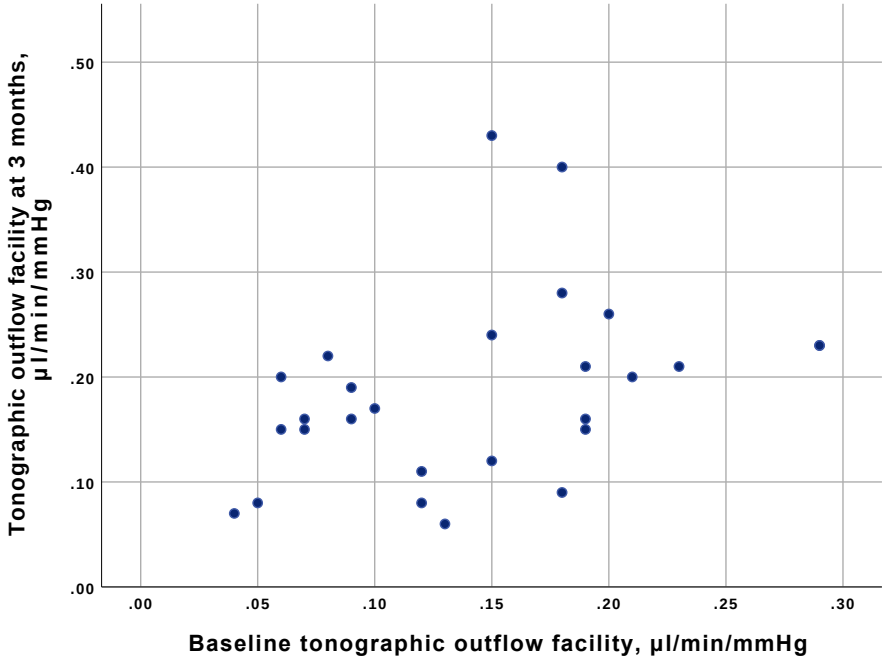
Whilst TOF in cataract group only improved at 3 and 6 months post operatively, in the glaucoma group only month 6 TOF enhancement was statistically significant. The comparative data are presented in Figure 3-5**.**

Figure 3-5. Demonstrates facility of outflow alteration during 12-month period in each group of patients separately. (The vertical bars represent range; the boxes outer border shows the25 and 75-percentiles and the thick middle line represents the median) statistically significant*



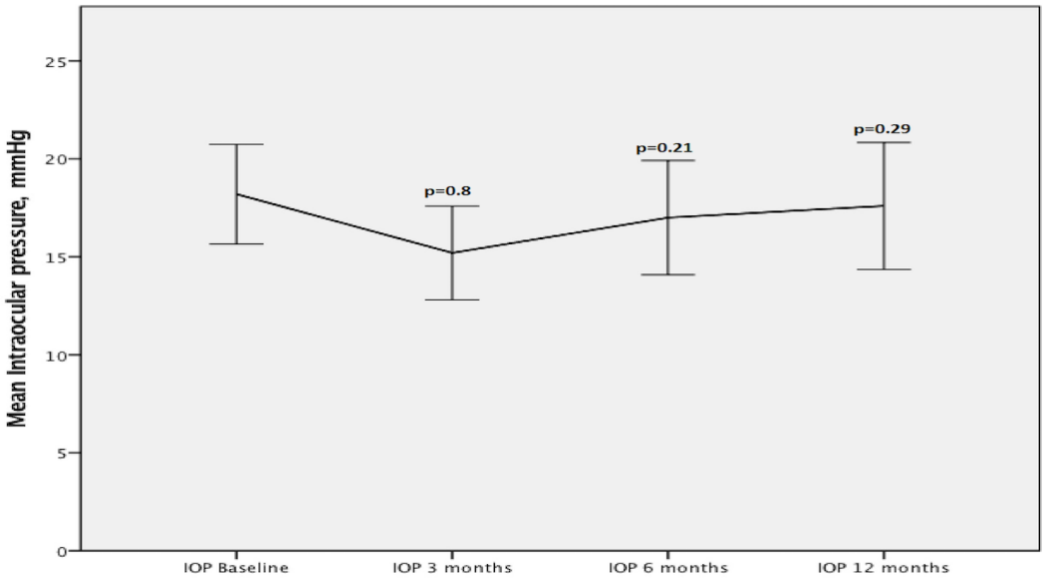
Overall baseline TOF was weakly correlated to TOF at 3-month post operatively (p=0.05, r=0.40) (Figure 3-6).

Figure 3-6. illustrates Pearson correlation on the scatter plot between baseline TOF and 3 months after surgery TOF. p=0.05, r=0.40



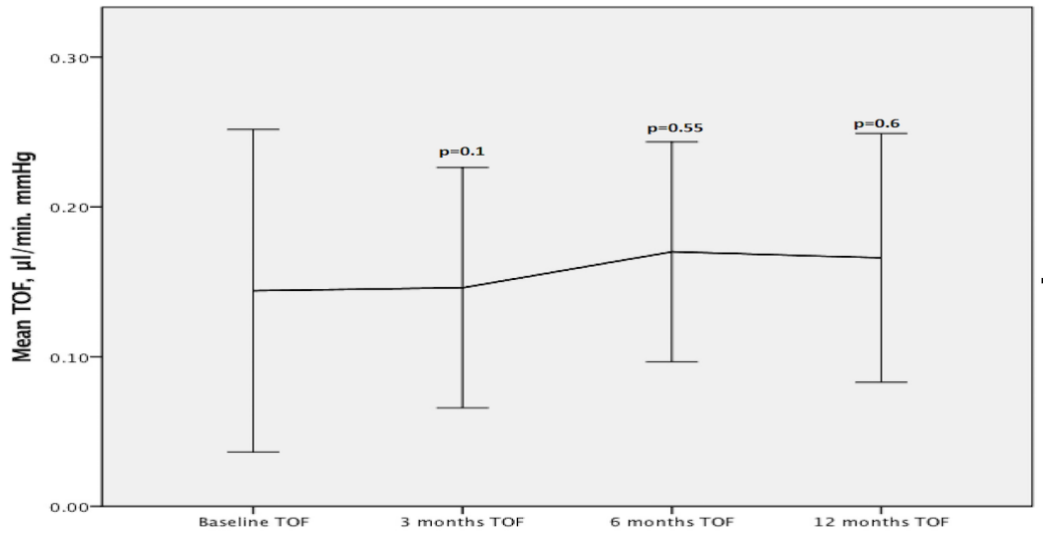
We identified five patients (with cataract) who did not undergo any intraocular surgery in their contralateral eyes during the follow up period and used their data as comparison. The average age of this group was 64.4±7.4 years. They all had open angles on gonioscopy (Shaffer grading of 3 or 4 in all quadrants) with mean anterior chamber depth of 2.98±0.62 mm. The baseline IOP for this group was 18.0±2.09 mmHg, which did not change significantly at 3, 6 and 12 months' visits (15.2±1.90 mmHg at 3 months, p=0.84, 16.4±2.07 mmHg, p=0.21 and 16.8±2.71 -mmHg at 12 months, p=0.29) (Figure 3-7).

*Figure 3-7. illustrates intraocular pressure changes during postoperative visits in the control group. * statistically significant*



Similarly, the facility of outflow at 3, 6 and 12 months compared with baseline, was not statistically different at any time point (0.18±0.08 vs 0.18±0.09 at 3 months, p=0.1, 0.18±0.09 at 6 months, p=0.55 and 0.18±0.07 µl/min/mmHg at 12 months, p=0.6) (Figure 3-8**).**

*Figure 3-8. demonstrates tonographic outflow facility changes during post-operative visits in the control group. * statistically significant*



Discussion

This is the first study to assess the facility of tonographic outflow after modern small incision phacoemulsification with intraocular lens implantation using electronic Schiøtz tonography. We demonstrated that IOP was significantly reduced at 3, 6 and 12 months after cataract surgery. In this study, the overall mean IOP reduction varied between 10 to 12%. There were also corresponding positive enhancement of TOF at all time points.

Numerous studies have explored the effect of phacoemulsification cataract surgery on IOP; all demonstrated significant but variable fall in IOP (1.5 - 9.0 mmHg) postoperatively (Slabaugh et al., 2014; Moghimi et al., 2017; Arthur et al., 2014; Shrivastava and Singh, 2010; Brooks and Gilles, 1992; Shingleton et al., 2006; Samuelson et al., 2011). However, current evidence is very limited on the effect of phacoemulsification on the facility of outflow.

In the Ocular Hypertension Treatment Study (OHTS) the IOP reduction was 4.0 mmHg three years after cataract surgery (Mansberger et al., 2012), which was greater than observed IOP reduction in our study at 12 months (2.0±3.6 mmHg). However, in the OHTS, baseline IOP was slightly higher (19.8±3.2 mmHg) compared to our cohort (16.0±3.7 mmHg). The IOP reduction after phacoemulsification has been shown to be proportional to the preoperative IOP, with significantly greater IOP reduction observed among those with higher

preoperative IOP (Pfeiffer et al., 2015); this may explain the greater IOP reduction in the OHTS group.

A recent randomised control trial of a Schlemm's canal micro stent (Hydrus II) with phacoemulsification in patients with open angle glaucoma, assessed 100 patients (Pfeiffer et al., 2015). Cases were randomised to have either cataract surgery (CS) alone or CS with micro stent implant. In the CS group, washed-out IOP reduced from baseline of 26.6±4.2 to 17.4±3.7 mmHg at 12 months. The mean IOP reduction was 9.0 mmHg at 1 year. This study had much higher baseline IOP (26.6±4.2 mmHg) and consequently, greater observed IOP reduction (9.0 mmHg) at one year, compared to our study (2.0±3.6 mmHg) and OHTS (4.0 mmHg) (Eid, 2011). One should take into consideration the effect of regression to the mean phenomenon when interpreting a physiological values such as IOP which is known to fluctuate (Pfeiffer et al., 2015).

Contrary to our study, Meyer and associates (Meyer et al., 1997) reported no enhancement of the facility of outflow at 6 weeks' post phacoemulsification cataract surgery (baseline outflow facility of 0.39 ± 0.23 vs 0.37±0.16 µl/min. mmHg 6 weeks). This may partly be attributable to the inconsistency of pneumatonography technique used in their study, highlighted by their much higher than usual baseline outflow facility value compared to other historical data (Chen et al., 2015).

Kee and Moon (Kee and Moon, 2000) carried out pneumatonography before and 2 months after phacoemulsification with intraocular lens implantation in 42 patients with cataract. In this study, the IOP reduction was 2.4±0.4 mmHg at 2-month post operatively. They demonstrated that outflow facility improved from 0.26±0.01 to 0.30±0.01 µl/min/mmHg 2 months after lens extraction. Although the duration of that study was short, their findings are in agreement with our results at 3 months (0.14±0.07 pre-operatively vs 0.18±0.09 µl/min/mmHg at 3-months). It is worth pointing out that the latter study did not report the anterior chamber depth or gonioscopy of their cases prior to the cataract surgery. One would assume that considering the higher prevalence of angle closure in the Korean population, narrow angle cases might have been included in their study inadvertently, making the effect of the lens removal on IOP reduction and the facility of outflow more exaggerated.

Tonographic outflow facility encompasses changes in trabecular outflow facility, as well as any pressure-dependent changes to inflow (pseudo-facility) or uveoscleral outflow (R. F. Brubaker, 2004; Kupfer and Sanderson, 1968). Nonetheless, in our study we investigated the change in TOF before and after cataract surgery using the same measurement technique, which theoretically means that these potential confounding effect should be less of an issue.

Although the most widely held view regarding the mechanism of IOP lowering effect post phacoemulsification is through increased trabecular outflow facility,

there were some speculations as to whether phacoemulsification may affect uveoscleral outflow as well as other aqueous humour dynamic parameters (Mathalone et al., 2005). In order to explore this, we made some calculations using the Goldmann's equation (see **Appendix**); If we assume that other aspects of aqueous dynamic parameters, such as aqueous production (*Ff*), uveoscleral outflow (*Fu*) or episcleral venous pressure (*Pe*) are unlikely to be affected by phacoemulsification and the mechanism of IOP lowering effect of phacoemulsification cataract surgery is solely via the increase in trabecular outflow facility, then according to Goldmann's equation, a 10-12% reduction in intraocular pressure (Pi) at all post op visits seen in our study should correspond to 11-18% increase in TOF (C) and this correlated well with our measured increase of 10-15% in TOF. As this largely accounts for the amount of IOP reduction, one can therefore conclude that the reason for the IOP drop post phacoemulsification is caused by an increase in trabecular outflow facility rather than any effects on other aqueous dynamic parameters such as uveoscleral outflow (see Appendix).

In our study, there was no linear correlation between axial length, IOP and TOF. The available evidence generally corroborates our findings with regards to axial length and IOP (Lee et al., 2004; Guan et al., 2013; DeVience et al., 2017). However, there are no previous studies looking at the relationship between TOF and axial length.

However, the question remains as to the underlying mechanism for the increase in trabecular outflow facility. Both mechanical changes as well as modifications at the cellular level have been put forward as the potential reason for the increase in outflow facility post phacoemulsification (Shingleton et al., 2006; Wang et al., 2003; Tsuboi et al., 2012; Mehdizadeh, 2008; Zhao et al., 2016). Although this is outside the scope of this study, it is useful to describe these theories briefly.

Based on the mechanical theory, the anterior chamber biometric alterations after phacoemulsification as well as other changes (Mehdizadeh, 2008; Zhao et al., 2016), may have been responsible for the increase in trabecular outflow facility (Shingleton et al., 2006). Zhao et al. exhibited a significant expansion of the Schlemm's canal based on optical coherent scans in eyes that had undergone phacoemulsification. Mehdizadeh also contemplated that the changes in the contractility of the ciliary muscles may have been accounted for the increase in trabecular outflow facility and the subsequent drop in IOP (Mehdizadeh, 2008).

Per the cellular theory, proposed by Wang et al (Wang et al., 2003) and Tsuboi et al.(Tsuboi et al., 2012), ultrasound can induce chemical and cellular changes *in vitro* in cultured trabecular meshwork cells. However, another *in vivo* clinical studies failed to show a linear correlation between ultrasound energy and IOP reduction after cataract surgery (Lee et al., 2016). Siriwardena et al. (Siriwardena et al., 2000) showed that there is a prolonged presence of flare in

the anterior chamber after cataract surgery. This suggests that aqueous-blood barrier breakdown after cataract surgery may contribute to trabecular meshwork changes postoperatively.

One limitation of our study is that POAG cases should have had a wash-out period from their glaucoma medications prior to study measurements. However, the number of medications which can affect trabecular outflow facility remained unchanged postoperatively. Furthermore, the results of the sub-group analysis were also similar to that of the combined data. However, because the numbers within each subgroup were small, the subgroup analysis should be interpreted with caution. Measurements collected from both eyes of an individual, are often correlated. Standard statistical tests such as *t test*, analysis of variance, confidence intervals and linear regression are only valid, if the parameters are independent (Sainani, 2010). Some studies have shown that including measurements from both eyes without adjusting for the correlation between two eyes have substantial effect on results (underestimates the standard error and generates falsely small p values) (Rosner, 1982). However, between-eye correlation can be exploited to include both eyes in some paired-eye studies (Beckman et al., 1990). The other approach is to take the average of measurements of two eyes (Newcombe and Duff, 1987; Murdoch, 1998). These approaches reduce information loss if the between-eye correlation is low. The choice of statistical approach depends on the research question. If the interest is

not eye specific, the individual level analysis might be the most appropriate method. In the present study I only include one eye per patient.

To our knowledge, this is the first study to assess the tonographic outflow facility after small incision phacoemulsification cataract extraction and IOL implantation using electronic Schiøtz tonography (which is the least variable measurement technique compared to other techniques); with documented anterior chamber depth and gonioscopy. We demonstrated that the IOP drop is accounted for by the increase in tonographic outflow facility. However, the exact mechanism of the outflow facility enhancement remains unclear from this study. Further research is needed to elicit this important question.

Chapter 4| The aqueous humour dynamics changes in uveitic eye

This chapter is presented as a published paper and is an exact copy of the following journal publication.

Pouya Alaghband, Alexander Jan Baneke, Elizabeth Galvis, et al. Aqueous Humor Dynamics in Uveitic Eyes**.** American Journal of Ophthalmology, 2019, 208, 347-355

Introduction

Uveitis is one of the most common ophthalmic conditions which is seen in eye departments globally. The prevalence of uveitis varies in different parts of the world (Acharya et al., 2013; Gritz and Wong, 2004; Suhler et al., 2008); however, idiopathic recurrent anterior uveitis (Chang and Wakefield, 2002; Miserocchi et al., 2013) is the most common diagnosis in those affected. One of the most serious sight threatening sequelae from this condition is uveitic glaucoma, which has a reported incidence of between 5 to 24% (Daniel et al., 2017; Sallam et al., 2009; Sng et al., 2012; Friedman et al., 2013) amongst long-term uveitic eyes. Despite the relatively high prevalence, there have only been a few previous aqueous humour dynamic studies exploring the pathogenesis of uveitic glaucoma in humans. This is partly due to measurement challenges which are unfortunately quite common in uveitic glaucoma/OHT cases. Firstly, eyes with prior intraocular surgery, such as cataract surgery, glaucoma filtration surgery or iridotomy/iridectomy, must be excluded. This is due to irido-lenticular barrier compromise which subsequently may lead to excessive

posterior flow of fluorescein and thereby disrupt the fluorescein loss assumptions underlying the fluorophotometry measurements (Brubaker and McLaren, 1985; Gulati et al., 2011). Secondly, active uveitis can render the fluorophotometry measurements inaccurate. This is because of the presence of excessive protein in the anterior chamber which can bind with the fluorescein molecule and disrupt its natural clearance from the anterior chamber (Brubaker and McLaren, 1985; Gulati et al., 2011).

Considering these limitations, it is not surprising that there have only been two previous human aqueous humour dynamics studies in uveitis or uveitic glaucoma/OHT and both were in those with active uveitis. The first study was by Ladas and associates (Ladas et al., 2001) who only investigated the correlation between tonographic outflow facility measured by Schiøtz tonography and laser flare photometry. However, they did not measure the aqueous flow rate. Their cohort comprised of patients with active uveitis (any type of uveitis including pan-uveitis and posterior uveitis were included). None of the eyes had any history of raised intra-ocular pressure (IOP). They showed that higher flare measurement in the anterior chamber coincided with lower outflow facility.

The other study by Johnson et al. (Johnson et al., 1983), compared tonographic outflow facility (Schiøtz) and aqueous flow rate (fluorophotometry) in 10 eyes with cyclitis to their fellow healthy eyes. The results showed that the aqueous flow rate and facility of outflow were not significantly different compared to

unaffected fellow eyes. Only three cases had raised IOP at the time of the measurement. They also had some anterior chamber activity (trace to 2+ cells and flare in the anterior chamber was reported) which may have compromised the accuracy of the tonographic outflow facility and aqueous flow rate measurements.

In regard to aqueous humour dynamics, there are probably three distinct circumstances where uveitis can lead to raised intra-ocular pressure; (1) those eyes that are currently actively inflamed, with anterior chamber inflammatory cells and proteins as well as concurrent steroid use; (2) previous severe or multiple uveitis attacks with a high likelihood of having had incisional ocular surgeries such as glaucoma filtration or cataract surgeries (such as juvenile chronic arthritis associated uveitis); (3) previous multiple but moderate anterior uveitis attacks but currently quiescent.

As fluorophotometry can be inaccurate in eyes that are actively inflamed, we have therefore designed this study specifically investigating quiescent eyes of the last category of the aforementioned list. This helps us understand why some eyes develop uveitic glaucoma/OHT after only a few attacks of anterior uveitis and not others.

Materials and methods

This study is a cross-sectional study comparing aqueous humour dynamics in age-matched healthy volunteers, previous anterior uveitis (without glaucoma) and those with uveitic glaucoma/OHT but no active uveitis at the time of study measurement.

Ethical approval for this study was obtained from the local St Thomas' Hospital research ethics committee. This research followed the tenets of the Declaration of Helsinki (http://www.clinical-trials.gov, identifier **NCT02765308, 6th** May 2016)**.** Healthy volunteers were recruited from the hospital staff and their family members after having a comprehensive ophthalmic examination. Patients with recurrent anterior uveitis were identified from our uveitis and glaucoma clinics at St Thomas' Hospital, London, United Kingdom. A patient information leaflet was provided at the initial contact, and signed informed consent was obtained before glaucoma eye drop washout and study measurements took place. Participants enrolled in the study were divided into three groups (1) previous recurrent anterior uveitis (≥ 3 attacks) with glaucoma/OHT (uveitic glaucoma) on topical glaucoma drops treatment (group 1); (2) previous recurrent anterior uveitis (≥ 3 attacks) with normal IOP and no diagnosis of glaucoma (group 2); and (3) healthy volunteers (group 3). All uveitic eyes were quiescent at the time of enrolment (defined by absence of any flare/cells on slit lamp biomicroscopy). All patients in the uveitic glaucoma/OHT group had a 4-week washout period

from their anti-hypertensive and steroid treatments prior to their study measurements (patients had a safety visit two-weeks after commencing the washout).

Eligibility criteria

Inclusion Criteria

- Age >18 years
- Adequate cognitive function and ability to understand verbal and written information in English
- Previous recurrent anterior uveitis (≥ 3 attacks) with/without OHT/glaucoma

Exclusion Criteria

- Other glaucoma diagnosis including pigment dispersion syndrome and pseudoexfoliation
- Active uveitis
- Ocular trauma
- Intraocular or keratorefractive surgery
- Use of systemic medication that may affect aqueous humour production such as beta-blockers
- A history of allergy or hypersensitivity to fluorescein
- Any abnormalities preventing reliable IOP or fluorophotometric readings

Primary outcome measures

- Tonographic outflow facility (measured by digital Schiøtz tonometry)

- Aqueous flow rate (measured by fluorophotometry)
- Uveoscleral outflow (calculated from the Goldmann's equation)

Measurements

All patients underwent clinical ophthalmic examinations including visual acuity, slit lamp biomicroscopy, gonioscopy, anterior chamber depth, and axial length measurement (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 ophthalmoscopy. 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 < 26 years, 5 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 (FM-2, Fluorotron Master ocular fluorophotometer; OcuMetrics, Mountain View, CA) from 9 AM to 12 noon. The aqueous flow rate was determined using dedicated software provided with the fluorophotometer. Triplicate scans were collected and repeated at 1-hour intervals for four measurements to determine the aqueous flow rate (Ff). Following each set of scans, IOP was measured using pneumatonometry (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 for each eye).

Tonographic outflow facility (*C*) was measured by constant weight tonography (5.5. 7.5 or 10 g) using a modified digital Schiøtz tonographer (designed by the Department of Bioengineering, Imperial College, London, UK) at 10 – 11 AM on the same day. Schiøtz tonography can affect the fluorophotometry by changing the fluorescence of the corneal epithelium; hence it is done towards the end of the study measurements. Our device used an original Schiøtz tonographer footplate from a commercially available unit (model 720, Berkeley Bioengineering Inc., San Leandro, CA, USA) attached to a 3D printed shell that was designed such that the weight conformed to the specifications set out by the Committee on Standardization of Tonometers (Friedenwald, 1954). Displacement of the weighted plunger was measured using a linear variable differential transformer (LVDT; MHR series, TE Connectivity, Schaffhausen, CH, USA) driven by a signal conditioner (AD698, Analog Devices, Norwood, MA, USA) and captured digitally by a data acquisition system (USB-6009, National Instruments, Austen, TX, USA). Validation studies confirmed that the LVDT voltage output was linear with respect to the Schiøtz scale reading (Figure 4-2), where each scale reading is equivalent to 0.05 mm of plunger displacement (Friedenwald, 1954). Facility was estimated using Grant's equation (Equation 2) (Grant, 1950).

$$C = \frac{V_{c,t} - V_{c,0} + \frac{1}{K}\left(log\ P_{t,0} - logP_{t,t}\right)}{\left(\frac{P_{t,0} + P_{t,t}}{2} - P_0 - \Delta P_v\right) t} \qquad Equation\ 2$$

where $V_{c,t}$ and $V_{c,0}$ are the aqueous volumes displaced at time t and at the start of tonography ($t = 0$). $P_{t,t}$ and $P_{t,0}$ are values of IOP at time t and at the start of tonography. P_0 is the IOP immediately prior to the start of tonography. ΔP_v is the change in episcleral venous pressure, assumed to be 1.25 mmHg (Friedenwald, 1948), and K is the coefficient of ocular rigidity, assumed to be 0.0215/µl^{-1} (Moses and Becker, 1958). $V_{c,t}, V_{c,0}$, $P_{t,t}$, $P_{t,0}$ and P_0 were determined based on the value of the Schiøtz scale reading and tables provided by Moses and Becker (Moses and Becker, 1958). By minimising the root mean square error between the tonographic tracing (

Figure 4-3) and Equation 1 , the optimal value of C was determined numerically. At present the clinical measurement of uveoscleral outflow in humans is not possible (Johnson et al., 2017); hence this value is generally calculated from the Goldmann's equation. Sit and McLaren used a computerized venomanometry to measure episcleral venous pressure (EVP) (Sit and McLaren, 2011; Brubaker, 1967). They illustrated that EVP in normal subjects can vary between 6 and 10 mmHg. Therefore, we have used this EVP range for our calculations. However, to make this calculation valid, it must be assumed that the episcleral venous pressure did not vary significantly between all three groups of patients in our study.

Uveoscleral outflow was calculated using Goldmann's equation (Equation 3) with an assumed episcleral venous pressure of 10 mmHg.

"*Ff*" is the rate of aqueous humour formation measured by fluorophotometry, "*C* "is the tonographic facility of outflow, "*Pi*" is the intraocular pressure, "*Pe*" is the episcleral venous pressure, and "*Fu*" is uveoscleral flow.

$$Ff = (Pi - Pe)C + Fu \qquad Equation\ 3$$

Therefore,

$$Fu = Ff - C(Pi - Pe)$$

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.

Sample size calculation

The sample size estimate (30 patients/group) is based on the results of paired measurements of the three parameters in a previous study done at the Mayo Clinic by KSL (Lim et al., 2008). Using these estimates, and the adjusted alpha (0.0167), detectable differences for each of the 3 primary parameters of interest were calculated and the results are given in the following table:

Variable	Coefficient of Variation	Detectable Difference	Standard deviation	Power
IOP	20%	5%	2.2	90%
Aqueous flow rate	25%	5.4%	0.8	90%
Facility of outflow	37%	7.5%	0.1	90%

(Alpha=0.0167, two-sided t test of paired samples, 30 subjects per group)

Data analysis

Histograms and 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 was used to compare continuous variables among groups. When data did not follow normality, non-parametric methods of analysis (Mann-Whitney U and Kruskal-Wallis tests) were used. Correlation coefficient analysis was used to determine the association of one parameter versus another parameter of aqueous humour dynamics. $P<0.05$ was considered statistically significant (all analyses, SPSS 24.0; SPSS, Chicago, IL).

Results

We screened one hundred and one patients between February 2014 and February-2017. Nine patients did not meet the inclusion criteria. Thirty patients with recurrent anterior uveitis and being treated for secondary glaucoma/OHT (group-1) and 32-patients with previous recurrent anterior uveitis (group 2) without OHT or glaucoma and with normal IOP who met the inclusion/exclusion criteria were recruited. Thirty healthy volunteers were enrolled as controls (group-3) over the same period. There was a female preponderance in groups 2 and 3. Most subjects were white Caucasians with a few African/Caribbean and Asians in groups 2 and 3 but a slightly higher presence of African/Caribbean patients in group 1 ($p=0.06$). The mean age in all 3 groups were comparable ($p=0.3$). The best corrected visual acuity in the uveitic glaucoma/OHT (group 1) was worse compared to other groups

(p=0.002). This was primarily due to lens opacity and severity of glaucoma in the group 1. Anterior chamber depth, axial length and central corneal thickness were similar across all groups (p=0.3). Visual field parameters were worse in group 1 compared to other two groups (p=0.05). All patients had open angles on gonioscopy with only five (17%) patients in group -1 with non-contiguous patchy peripheral anterior synechiae. The subjects baseline characteristics are summarised in Table 4-6. Baseline characteristics of all participants are shown.

*Table 4-6. Baseline characteristics of all participants are shown. Mean±SD is presented for each parameter. *statistical significance*

		Comparison between Group 1 and Group 3			Comparison between Group 1 and Group 2			Comparison between Group 2 and Group 3	
	Group 1 [uveitic glaucoma/ OHT] (n=30)	P value	95% CI	Group 2 [uveitis] (n=32)	P value	95% CI	Group 3 [Normal] (n=30)	P value	95% CI
Gender (F: M)	14(47%): 16(53%)	0.06	---	24(80%): 8(20%)	0.06	---	20(67%): 10 (33%)	0.06	---
Ethnicity (W: B: A)	10: 13: 7	0.01	---	22: 9: 1	0.01	---	21: 5: 4	0.01	---
Age, years	50±14.0	0.9	-7.2-8.4	49±11.1	0.4	-12.3-3.4	44±13.6	0.3	-12.8-2.7
BCVA, LogMAR	0.04±0.2	**0.02***	-0.2- -0.01	-0.06±0.1	**0.002***	-0.2- -0.04	-0.09±0.1	0.7	-0.1-0.05
CCT, µm	552±37.0	0.6	-27.5-11.6	544±31.2	0.7	-26.0-13.8	546±28.2	0.9	-17.7-21.4
ACD, mm	3.34±0.3	0.6	-0.3-0.12	3.26±0.4	0.3	-0.1- 0.4	3.49±0.3	0.06	-0.02-0.4
AXL, mm	23.4±4.0	0.8	-1.2-1.9	23.8±1.4	0.5	-0.8- 2.3	24.2±1.5	0.8	-1.2-1.9
MD, dB	-3.79±6.1	0.1	-0.3-4.3	-1.80±2.4	**0.05***	0.03-4.8	-1.36±1.8	0.9	-1.9-2.7

F: female, M: male, W: white, B: black, A: Asian, BCVA; best corrected visual acuity, CCT: central corneal thickness, ACD: anterior chamber depth, AXL: axial length, MD: mean deviation, CI: confidence interval

Mean IOP was significantly higher in the uveitic glaucoma/OHT group compared to the other two groups ($p<0.001$). The tonographic outflow facility (C) was markedly lower in the uveitic glaucoma/OHT group compared to the other two groups ($p=0.005$).

However, aqueous flow rate was not statistically different between the three groups (2.47±0.9 (group 1) vs 2.18±0.9 (group 2) vs 2.32±0.8 (group 3) µl/min, $p=0.3$). Additionally, no significant difference in uveoscleral outflow (with assumed episcleral venous pressure of 10 mmHg based on Sit and McLaren's work (Sit et al., 2011)) was detected between the three groups ($p=0.9$). The full aqueous humour dynamic results are shown in Table 4-7.

*Table 4-7. Aqueous humour parameters and comparison made between all 3 groups. Mean±SD is presented for each parameter. *statistical significance*

		Comparison between Group 1 and Group 3			Comparison between Group 1 and Group 2			Comparison between Group 2 and Group 3	
	Group 1 [Uveitic glaucoma/OHT] (n=30)	P value	95% CI	Group 2 [Uveitis] (n=32)	P value	95% CI	Group 3 [Normal] (n=30)	P value	95% CI
IOP, mmHg (min-max)	25±10.2 (10-52)	**<0.001***	-12.5--4.6	16±2.5 (10-22)	**<0.01***	4.3-12.5	16±2.2 (11-19)	0.9	-3.6-4.7
Aqueous flow rate(Ft), µl/min	2.47±0.9	0.3	-0.8-0.21	2.18±0.9	0.7	-0.66-0.37	2.32±0.8	0.7	-0.36-0.65
Trabecular outflow facility(C), µl/min/mmHg	0.18±0.1	**0.005***	0.02-.15	0.27±0.1	**0.04***	0.01-0.14	0.25±0.1	0.7	-0.08-0.04
Uveoscleral outflow (Fu), mmHg	0.49±1.6	0.9	-0.73-1.02	0.64±1.3	0.7	-0.62-1.15	0.75±1.4	0.9	-0.75-0.99

IOP: intraocular pressure, OHT: ocular hypertension. FU: calculated uveoscleral outflow assumed Episcleral venous pressure of 10 mmHg

A break-down of different uveitis diagnoses in groups 1 and 2 are provided in Table 4-8. Most cases were idiopathic anterior uveitis.

Table 4-8. The uveitis diagnosis in groups 1 and 2 are shown here

	Idiopathic	Sarcoidosis	HLA-B27 associated	Psoriasis	Tuberculosis related	Herpetic
Group 1 (n=30)	21	4	3	0	1	1
Group 2 (n=32)	22	2	7	1	0	0

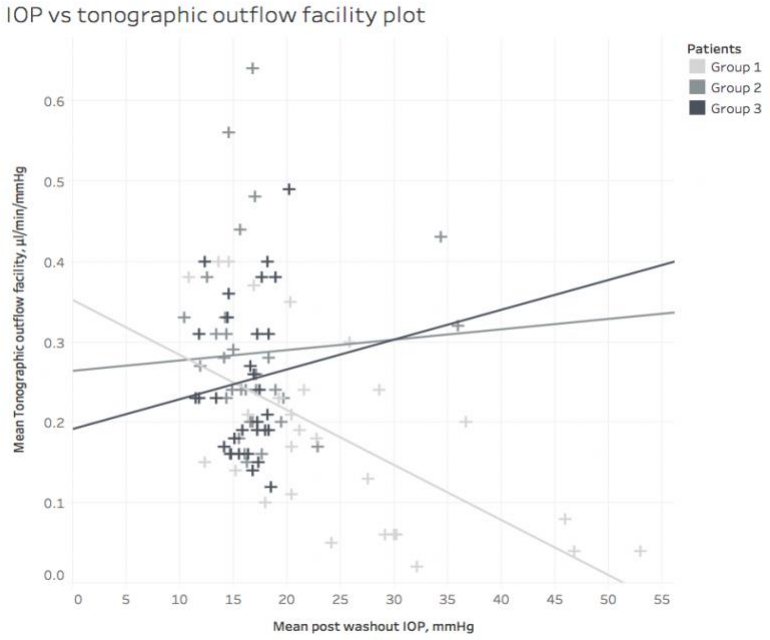
The study eyes in the group 1 on average had 4.5 episodes (range 3-6) of uveitis attacks whilst eyes in the group 2 had on average 6 episodes (range 3-10) prior to study measurements.

If, rather than including all individuals in the uveitic glaucoma/OHT group as in Table 2, we excluded 14 individuals who maintained a normal IOP (< 21 mmHg) post washout, the average IOP in the uveitic glaucoma/OHT group increased to 31.8±9.7 mmHg, which was greater than either the uveitis group 2 (16±2.5 mmHg) and control group 3 (16±2.2 mmHg; $p<0.001$). Correspondingly, the tonographic outflow facility in the uveitic glaucoma/OHT group excluding those with normal IOP post-washout was 0.13±0.1 µl/min/mmHg, which was significantly lower than either the uveitis group 2 (0.27±0.1 µl/min/mmHg) or control group 3 (0.25±0.1 µl/min/mmHg; $p<0.001$). Nonetheless, even after removing those with normal post-washout IOP, there was still no statistically significant difference in aqueous flow rate

between three groups (2.42±0.9 (group-1) vs 2.18±0.9 (group 2) vs 2.32±0.8 µl/min (group 3), p=0.4). Similarly, the uveoscleral outflow was comparable between all groups (-0.01±2.06 vs 0.64±1.3 in uveitis (group 2) vs 0.75±1.4 in controls (group 3), p=0.1).

Correlation was made between IOP and aqueous flow rate, tonographic outflow facility, uveoscleral outflow, ACD or AXL. Only the correlation between post-washout IOP and tonographic outflow facility was statistically significant with a strong negative correlation observed in group 1 (R^2=0.86, p<0.001). The other correlations were not significant (p>0.09) (Figure 4-1). However, correlation between IOP and aqueous humour parameters was not significant in other groups (P=0.6).

Figure 4-1. Intraocular pressure (IOP) vs tonographic outflow facility plot. Correlation was made between post washout IOP and tonographic outflow facility. Only the correlation between post washout IOP and tonographic outflow facility was statistically significant with a strong negative correlation observed in group 1 (R^2=0.86, p<0.001)[shown in light gray]. The other correlations were not significant (p>0.09). (Outlier were trimmed for the analysis).



Discussion

This is the first aqueous humour dynamic study in patients with uveitic glaucoma/OHT and recurrent anterior uveitis compared with age-matched healthy controls. However, one should be mindful of the fact that uveoscleral outflow was calculated by the Goldmann formula using assumed episcleral venous pressure. Additionally, we utilised indirect techniques to measure the aqueous flow rate and tonographic outflow facility. Consequently, these parameters may have been compromised by subclinical inflammation. The uveitis and uveitic glaucoma/OHT cases were all clinically quiescent at the time of enrolment and none in the uveitic glaucoma/OHT group had more than six previous anterior uveitis attacks. We demonstrated that the elevated intraocular pressure seen in the uveitic glaucoma/OHT eyes was due to reduced tonographic outflow facility (this is used as a proxy of measuring trabecular outflow facility (Moses et al., 1985)). The aqueous flow rate was not detectibly different amongst the 3 groups nor did the calculated uveoscleral outflow demonstrated any difference between three groups.

This study is unique in several aspects. Firstly, it encompasses age-matched healthy controls as well as those with previous recurrent anterior uveitis with or without glaucoma/OHT. Additionally, in this study, previously treated uveitic glaucoma/OHT patients underwent a 4-week washout period from their glaucoma medications, mydriatics and steroids before the study measurements. It is therefore suggestive that the reason for raised intraocular pressure, after

moderate number of recurrent anterior uveitis attacks, is caused by increased tonographic outflow resistance without significant impairment to aqueous production.

Although the reduction in tonographic outflow facility in our finding should not come as a surprise (as in almost all other types of glaucoma, tonographic outflow impairment is the primary cause of raised IOP (Brubaker, 1991), this is the first study to confirm this in uveitic glaucoma. Ladas and associates (Ladas et al., 2001) investigated the correlation between outflow facility measured by Schiøtz tonography and laser flare photometry in patients with active uveitis. They demonstrated that the higher the measured flare in the anterior chamber (>20 photon units/msec), the lower the outflow facility (0.21±0.12 µl/min/mmHg). They also reported that patients with flare <20 photon units/msec had a similar outflow facility to normal controls. Although increased aqueous protein level may lead to obstruction of trabecular meshwork pores in the acute phase, it may also have lasting effect on outflow facility as demonstrated by Epstein et al. (Epstein et al., 1978). They explored the facility of outflow in enucleated human eyes by infusing the eyes with human plasma and showed that facility of outflow reduced by over 40% and interestingly this was not resolved by irrigating the eyes with balanced salt solution. The authors speculated that this may be due to adhesion of serum components of plasma to the aqueous outflow system i.e. trabecular meshwork.

All uveitic glaucoma/OHT eyes in our cohort had clinically quiescent anterior chamber on slit lamp examination (although they may had subclinical inflammation; we did not have access to flaremeter to measure this) at the time of measurements; Nonetheless, there might have been lasting damage to TM due to repeated anterior chamber inflammation or even previous long-term use of topical steroids which can eventually cause compromised trabecular-outflow and consequently raised IOP. Mechanical obstruction due to peripheral anterior synechiae could also account for some of the reduced tonographic (trabecular) outflow facility. The evidence from animal studies suggests that inflammatory cells can cause blockage of trabecular outflow facility by simply clogging the trabecular meshwork pores (Rao et al., 1979). Chronic inflammation of the trabecular meshwork may lead to scar formation and permanent damage to the underlying tissue (Moorthy et al., 1997). In a recent multicentre study of risk factors of ocular hypertension in non-infectious uveitis (Daniel et al., 2017), the presence of peripheral anterior synechiae (PAS) carried a three-fold increased risk of developing OHT. Whilst in our study only 17% of uveitic glaucoma/OHT cases had some degree of non-contiguous PAS and none had more than 180 degrees of PAS, suggesting that raised IOP might have been due to micro-structural damage to the trabecular meshwork. Additionally, extracellular matrix accumulation in the trabecular meshwork or increased extension of the endothelial basement membrane along Schlemm's canal coincide with long-term use of steroids may play a part in obstruction of

trabecular outflow and subsequently raised intraocular pressure (Johnson et al., 1997; Overby et al., 2014).

Calculated uveoscleral outflow in our present study in human uveitic glaucoma/OHT (as a non-invasive direct clinical measurement remains elusive) is in marked contrast to uveoscleral outflow measured in monkeys' eyes with active uveitis by Toris and Pederson (Toris and Pederson, 1987). In their study using cynomolgus monkeys, the anterior uveitis was artificially induced with intra-cameral injection of albumin. The aqueous flow rate and uveoscleral outflow was measured using fluorescein isothiocyanate (FITC) dextran 70. They found that the uveoscleral outflow was four times greater in the inflamed eyes than the controlled eyes. In actively inflamed monkey eyes, ciliary body becomes oedematous and supra-choroidal space is enlarged and there is surge in the release of endogenous prostaglandins (Yousufzai et al., 1996). Therefore a transient increase in uveoscleral outflow is observed which subsides once the inflammation has settled as in our study. Based on this observation, the authors would like to speculate that topical prostaglandin is more likely to be effective in lowering IOP in quiescent eye than in active uveitis eye (Bhattacherjee, 1989).

Post-operative hypotony after glaucoma filtration surgery in uveitic glaucoma has been routinely attributed to 'aqueous shutdown' without any evidence to substantiate this claim (Da Mata et al., 1999; Rumelt, 2013; Tran et al., 2000). Therefore, one of the most interesting findings in our study is the similar level

of aqueous flow rate (in the absence of active inflammation) in all three groups. This suggests that after less than seven attacks of anterior uveitis, there may not be any significant damage to the ciliary epithelium and other associated apparatus involved in the production of aqueous in human eyes. We limited our uveitis groups to only those eyes with more than three attacks of anterior uveitis and interestingly, none of our recruited cases in group 1 had more than six attacks of uveitis. We identified many other cases of uveitic glaucoma with more than 6 previous attacks, but none were eligible for our study due to previous intraocular surgeries, such as cataract and glaucoma surgeries. It is therefore plausible that uveitic glaucoma patients who have more than six attacks of uveitis may have different aqueous dynamic parameters, including aqueous production rate change.

Based on available evidence and our own study, aqueous flow rate is probably only reduced in those cases of severe acute uveitis or those eyes with previous multiple (more than 6) and severe uveitis attacks, such as those associated with idiopathic juvenile arthritis. Therefore, surgical techniques (Gedde et al., 2018), rather than 'aqueous shutdown' are the most likely cause of hypotony post--glaucoma filtration surgeries, in those glaucoma eyes with moderate uveitis. However, it is difficult to substantiate this because of the limitations of current aqueous humour techniques in eyes which have undergone previous glaucoma surgery.

As part of the wash-out process before the aqueous humour dynamics measurements, we also observed an interesting finding in our study which may have significant clinical ramifications. After washout, the mean IOP in nearly half (47%) of the uveitic glaucoma/OHT group was less than 22 mmHg. It is likely that following a period of inactivity of the uveitis as well as cessation of topical steroid treatment, in those cases of presumed uveitic glaucoma/OHT, IOP may revert to normal after wash-out. Clinically, it is therefore sensible to consider treatment washout in this group of patients after these eyes have been controlled on glaucoma drops treatment after 6-12 months without any recurrence of their uveitis.

There are, however, a few inherent limitations in aqueous humour dynamics studies. The most important of all is that the eyes undergoing fluorophotometry measurements should not have had any intraocular surgery such as cataract surgery or iridotomy, which may compromise the irido-lenticular barrier to the posterior flow of fluorescein during fluorophotometry measurement (Brubaker and McLaren, 1985; Gulati et al., 2011). Active uveitis also renders the fluorophotometry measurement inaccurate due to the presence of excessive protein in the anterior chamber which can bind to fluorescein molecules. Furthermore, with the breakdown of the blood-aqueous barrier, fluorescein can diffuse through unconventional pathways, potentially distorting the assumptions about the standard diffusional loss of fluorescein during fluorophotometry

(Brubaker and McLaren, 1985; Gulati et al., 2011). Tonographic outflow facility is influenced by 'Pseudofacility' especially in uveitis due to possible subclinical inflammation (Moses et al., 1985). Therefore, there might be a discordance between tonographic outflow facility and trabecular outflow facility. Another issue relates to the measurement of episcleral venous pressure and uveoscleral outflow. At present the precise measurement of uveoscleral outflow is not possible in humans (Johnson et al., 2017); hence this value is generally calculated from the Goldmann's equation. As we are also unable to accurately measure the episcleral venous pressure (EVP)(Sit et al., 2011; Brubaker, 1967) despite some experimental methods of measuring EVP (Sit et al., 2011), they are not widely available. Therefore, it is generally accepted that EVP to be approximately 10-mmHg in humans. To make this calculation valid, it must be assumed that the EVP in these three groups of patients did not vary significantly. We did not perform flare measurement in our patients as we did not have the flare meter in our department; however, as we have taken great care in excluding any eyes with active uveitis, we do not believe that this measurement will affect the main findings of our study.

In summary, to our knowledge this is the first aqueous humour dynamic study in patients with previous recurrent anterior uveitis and uveitic glaucoma/OHT compared with age-matched healthy controls. We have demonstrated that elevated intraocular pressure seen in the uveitic glaucoma/OHT eyes (after less than seven previous attacks of uveitis) was due to reduced tonographic outflow

facility alone. The aqueous humour flow rate was not detectibly different among the three groups nor did the calculated uveoscleral outflow demonstrate any detectible difference between the three groups. Clinicians should also consider treatment washout for those with medically treated uveitic glaucoma in the future. However, future studies should be undertaken once we have better techniques of aqueous dynamics measurements in eyes with active uveitis and previous intraocular surgeries as well as non-invasive and accurate techniques for measuring EVP and uveoscleral outflow.

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Ellex	SLT	Research Grant, Lecture Fees
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Figure 4-2 Displacement calibration of the digital Schiøtz tonographer. Using a micrometre, the plunger of the LVDT was moved in increments of 50 µm, equivalent to 1 Schiøtz scale reading, over the range of -1 to 20 scale readings, whilst measuring the LVDT voltage output (supplemental Figure 1). Each position was measured 3 times, with all data shown. The voltage-displacement relationship was linear over the full range (R^2 = 0.9997), allowing the measured LVDT voltage to be converted into a Schiøtz scale reading. A scale reading of -1 is defined as the reading when the footplate is placed on a rigid spherical surface with a 15 mm radius of curvature (Friedenwald, 1954).

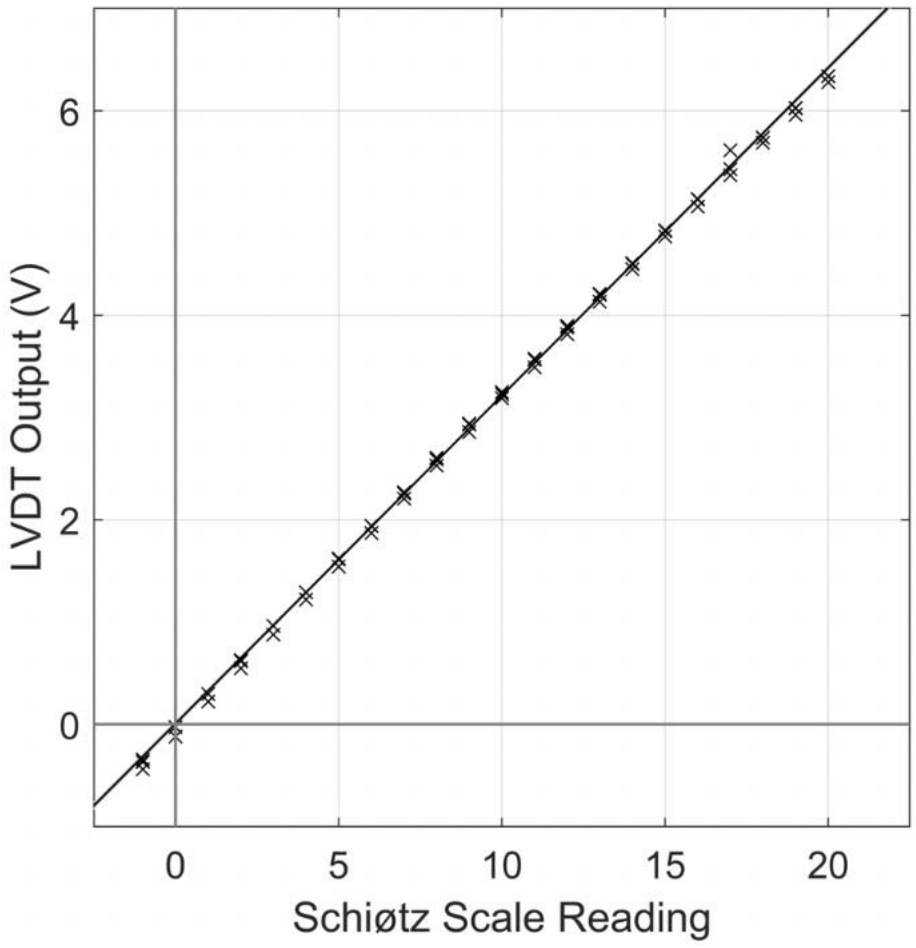
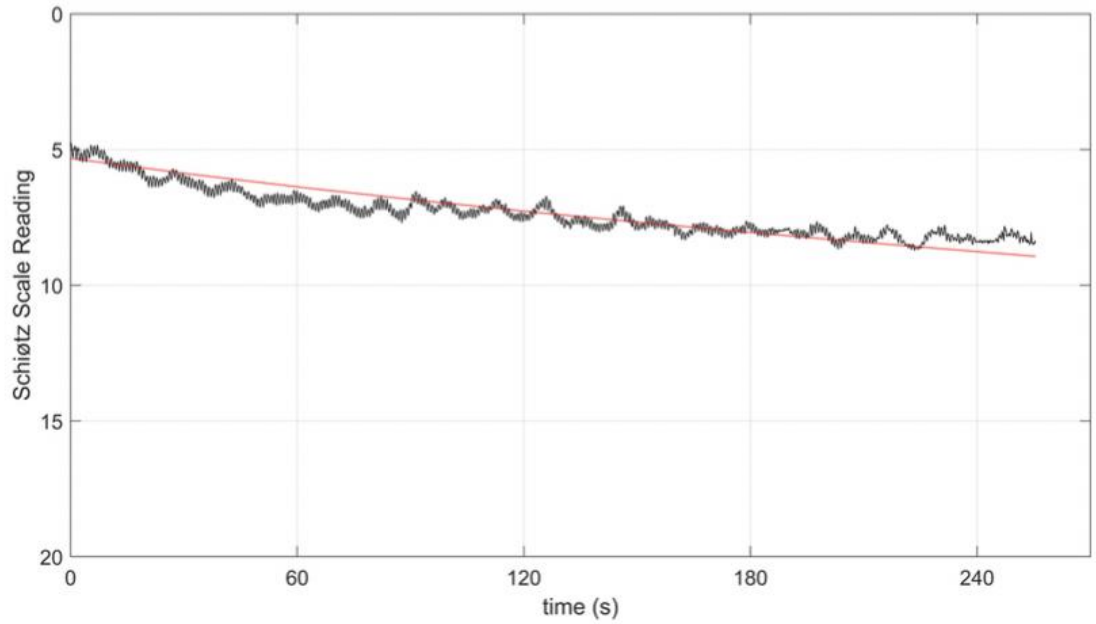


Figure 4-3 sample tonography tracing fit to Equation 1. The black trace shows the captured signal from the LVDT, converted into Schiøtz scale reading (see Figure 4-2). The red curve shows the predicted scale reading based on fitting Equation 1 to the black tracing for the optimal value of *C* (in this case 0.31 µl/min/mmHg). Tonography was performed with a 5.5--gram weight placed on the right eye of a uveitic patient without or ocular hypertension (IOP= -16.4 mmHg) glaucoma. The high frequency oscillations (~1 Hz) observed in the black tracing reflects the ocular pulse.



Chapter 5| The effect of high intensity focused ultrasound on aqueous humour dynamics

This chapter is presented as a published paper and is an exact copy of the following journal publication.

Pouya Alaghband, *Elizabeth Galvis, Alba Ramirez, et al. The Effect of High-Intensity Focused Ultrasound on Aqueous Humor Dynamics in Patients with Glaucoma***.** *Ophthalmology Glaucoma 2020; 3 (2), 122-129*

Introduction

Cyclodestructive procedures for glaucoma were introduced into clinical practice nearly a century ago (Vogt, 1936). Their primary aim is to ablate the ciliary epithelium which produces aqueous humour to reduce intraocular pressure (IOP). Various modalities have been used to achieve this goal, including: diathermy (Vogt, 1936), surgical excision (Verhoeff, 1924), cryotherapy (Beckman et al., 1972), and laser (Beckman and Sugar, 1973). Laser is now the most popular treatment method, using 810 nm diode laser, delivered either as trans-scleral diode photocoagulation (TSCP) (Weekers, 1924; Kosoko et al., 1996) or endoscopically via endocyclophotocoagulation (ECP) (Chen et al., 1997; Charles, 1981).

The other treatment option is to utilize ultrasound energy to modulate ciliary epithelium function. High-intensity focused ultrasound (HiFU) was first used to treat brain pathologies, such as Parkinson's disease in 1940s (Lynn and Putnam, 1944). The theoretical advantage of this technology is to have the treatment area focused on a well-defined section at a pre-set depth, thus limiting damage to

surrounding tissues (Lynn and Putnam, 1944). This technology was later used in ophthalmology practice to treat glaucoma in 1980s. Coleman and his colleagues (Lizzi et al., 1984; Coleman et al., 1986) conducted first studies using a commercially available device called Therapeutic Ultrasound System (Sonocare-Inc., Ridgewood, New Jersey, USA). They evaluated the efficacy and safety of high-intensity focused ultrasound (HiFU) in patients with uncontrolled IOP and advanced glaucoma (Coleman et al., 1985; Lizzi et al., 1984) with reasonable results. Similar outcome was later confirmed by another clinical study by Sterk et al. (Sterk et al., 1989). However, despite the encouraging initial evidence, the significant risk of complications, such as scleral staphyloma and perforation, corneal thinning, persistent hypotony, phthisis bulbi, and loss of visual acuity, led to abandonment of the procedure in the middle of 1990s (Maskin et al., 1989; Sterk et al., 1992, 1989). Additionally, the bulky design and complexity of the procedure were amongst other reasons for not pursuing this procedure further (Muratore, 2005). By refining the transducer design and the modes of energy delivery, Eye Tech Care (Rillieux- la-Pape, France) introduced a new ultrasound cycloplasty device using HiFU technology, called the EyeOP1 in 2011 (Charrel et al., 2011). This device has received CE mark in 2011 and Chinese FDA (CFDA) approval in 2017. The device delivers ultrasound energy to the ciliary body with pre-set parameters. This device consists of a console, treatment probe and the coupling cone. The probe consists of 6 miniature piezoelectric curved areas inside the

probe which create well circumscribed areas of treatment on the eye. The high frequency enables focused delivery of ultrasound. The focusing area is not more than 0.1 by 1 mm^2. The device pre-set parameters are 21 MHz frequency and 2.45 W acoustic power with activation period of 8-second. The HiFU probe is supplied in three sizes (11, 12, and 13-mm), which fit most ocular sizes, and for every patient, the choice of the right size is based directly on white-to-white and axial length measurements (Denis et al., 2015).

Initial reports suggested that HiFU has a comparable mechanism of action as other cyclodestructive procedures such as trans-scleral diode photocoagulation (TSCP) which lowers IOP by destroying ciliary processes and suppressing aqueous production (Denis et al., 2015; Aptel et al., 2010). However, Coleman-et-al. (Coleman et al., 1997) speculated that the focused ultrasound may have dual effects in reducing IOP by increasing outflow through the sclera from the induced scleral thinning. Mastropasqua et al. (Mastropasqua et al., 2016), in a study used anterior segment optical coherence tomography (AS-OCT) and in-vivo confocal microscopy. They indirectly assessed the uveo-scleral outflow pathway via scleral changes (as a surrogate for uveo-scleral outflow pathway) post HiFU treatment in glaucoma patients. They found an increased intra-scleral hyporeflective spaces in AS-OCT. Authors contemplated that these findings were likely led to an increase in uveo-scleral outflow.

The aqueous humour dynamics effects of HiFU treatment has never been studied before. We have designed this case-control study assessing the effects of HiFU on aqueous humour parameters, to investigate the mechanism of action.

Materials and methods

Ethics approval for this study was obtained from the National Health Service (NHS) research ethics committee, United Kingdom. This research conformed to tenets of the Declaration of Helsinki (https://Clinicaltrials.gov identifier is NCT02839590. The NIHR portfolio registration number of this study is 35357). Consecutive patients with glaucoma or ocular hypertension (OHT) (glaucoma was defined as open angle glaucoma cases including pigmentary glaucoma diagnosed based on abnormal visual field testing and corresponding disc changes diagnosed by a glaucoma specialist) and sub-optimal IOP control despite maximum medical treatment, were invited to participate in the study. The rationale for including this group of patients was that HiFU is effective in managing refractory glaucoma (Melamed et al., 2015; F Aptel et al., 2016). A patient information leaflet was provided at the initial contact, and signed consent was obtained before the measurements and treatment were carried out.

Eligibility criteria

All of the following inclusion criteria were met:

- All patients between the ages of 18 and 90 years
- Diagnosis of open angle glaucoma or OHT with sub-optimal IOP control despite maximum medical treatment
- Ability to undergo accurate fluorophotometry and tonography

Patients were excluded if they had any of the following:

- Mental impairment conflicting with informed consent or follow-up
- Allergy to fluorescein
- Current use of any investigational drug, device or current participation in an interventional clinical trial
- Previous intraocular incisional surgeries including iridotomies, cataract surgeries or glaucoma filtration surgery

All patients underwent a comprehensive ophthalmic examination including visual acuity, slit lamp examination, gonioscopy, anterior chamber depth, and axial length (IOL Master; Carl Zeiss Meditec Inc., Dublin, CA) measurement, central corneal thickness measurement (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 ophthalmoscopy.

Patients then underwent a 4-week wash-out period from all glaucoma treatments prior to baseline study measurements (patients had a safety visit, 2 weeks

post-washout). The washout process was repeated at 3-month visit, prior to study measurements (with a safety 2 weeks post washout visit).

The night before the baseline study visit (10 PM) for 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 and younger, 5 drops; age 26–35 years, 4 drops; 35 years of age and older, 3 drops) (Brubaker et al., 2001). 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, USA). 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 pneumatonometry (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 two eyes.

Tonographic outflow facility (*C*) was measured by constant weight tonography (5.5 -10 g) using a modified digital Schiøtz tonographer (designed by the Department of Bioengineering, Imperial College London, London, UK) at 10 – 11 AM. Our device used an original Schiøtz tonographer footplate from a

commercially available unit (model 720, Berkeley Bioengineering Inc., San Leandro, CA, USA) attached to a 3D printed shell that was designed such that the weight conformed to the standards set out by the Committee on Standardization of Tonometers (Friedenwald, 1954). Displacement of the weighted plunger was measured using a linear variable differential transformer (LVDT; MHR series, TE Connectivity, Schaffhausen, CH, USA) driven by a signal conditioner (AD698, Analog Devices, Norwood, MA, USA) and captured digitally by a data acquisition system (USB-6009, National Instruments, Austen, TX, USA). Validation studies (Alaghband et al., 2019) confirmed that the LVDT voltage output was linear with respect to the Schiøtz scale reading, where each scale reading is equivalent to 0.05 mm of plunger displacement. The procedure was then repeated (after 10 minutes) on the contralateral eye, with patching of the already tested eye (to prevent the cornea from drying out).

At present the clinical measurement of uveoscleral outflow in humans is not possible (Johnson et al., 2017) ; hence this value is generally calculated from the Goldmann's equation. Sit and McLaren (Sit et al., 2011) used a computerized venomanometry to measure episcleral venous pressure (EVP). They illustrated that EVP in normal subjects can vary between 6 and 10 mmHg. Therefore, we used this EVP range for our calculations. Uveoscleral outflow was calculated

using Goldmann's equation (Equation 3) with an assumed episcleral venous pressure of 10 mmHg.

"*Ff*" is the rate of aqueous humour formation measured by fluorophotometry, "*C* "is the tonographic facility of outflow, "*Pi*" is the intraocular pressure, "*Pe*" is the episcleral venous pressure, and "*Fu*" is uveoscleral flow (Equation 4).

$$Ff = (Pi - Pe)C + Fu.$$

Equation 3. Goldmann equation

Therefore

$$Fu = Ff - C(Pi - Pe)$$

Equation 4. modified Goldmann equation

HiFU Treatment

Three different probe sizes are available to account for differences in ocular anatomy. The probe size is determined for each patient, by optical biometry performed at baseline visit (measuring white-to-white diameter). A nomogram has been developed to facilitate white-to-white measurement to work out the probe size.

A coupling cone made of a polymer is placed on the eye. This is to ensure the centration and distance from the eye is maintained throughout the procedure. At the base of the probe there is a suction cup to create low level vacuum (225-mmHg) to stabilise the cup on the eye. The 4-ml cavity that is created between the eye, cone and treatment probe is filled with sterile saline solution at room temperature (BSS, Alcon Inc., Fort Worth, TX, USA, or equivalent

product). The six elliptical cylinder-shaped impacts are centred on an 11–13 mm diameter circle, depending on the ring diameter chosen, and spread over the eye circumference, while avoiding the nasal–temporal meridian. We used second-generation probe. This differs from the original version in its broader active transducer area (4 mm instead of 2.5 mm) and more precise temperature calibration of each single transducer. Other enhancements of the second-generation probe include optimised suction and centring on the eye globe; improved coupling of ultrasound due to removal of air bubbles in the liquid which could disturb the ultrasound beam; optimised ergonomics and improved clip to attach the probe into the cone.

All HiFU procedures were performed by an experienced surgeon (KSL) under peribulbar anaesthesia (a mixture of lidocaine 2% and levobuprocaine 7.5%). However, the procedure can be carried out under subtenon anaesthesia too. Post-operatively, patients were treated topically with dexamethasone 0.1% preservative free 2 hourly for 2 weeks and then qds for 2 weeks. Hypotensive medications were stopped immediately postoperatively on the discretion of the treating clinician.

Post op visits

Patients were reviewed at 1 week, 1 month and then 3 months post operatively. The hypotensive topical drops may have been resumed at the discretion of the treating clinician. There was no washout at 1 week and 1-month post op.

However, all hypotensive treatments were stopped for 4 weeks prior to 3 months' aqueous humour dynamic measurements (patients had a 2-week safety visit after commencing the washout).

Only one eye treated with HiFU (the worse eye affected by glaucoma/OHT) per participant was included in the data analysis.

Primary outcome measures

- Intraocular pressure (IOP)
- Facility of tonographic outflow (measured by digital Schiøtz tonography)
- Aqueous flow rate (measured by fluorophotometry)
- Uveoscleral outflow (calculated from the Goldmann's equation)

These parameters were measured pre-treatment (after four weeks' glaucoma treatment washout) and then at 3 months post-surgery (after four weeks' glaucoma medication treatment washout). Measures were taken from the operated eye and the contralateral non-operated eye which was used as the control.

Statistical analyses

Histograms and 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 was used to compare continuous variables among groups. When data did not follow normality, non-parametric methods of analysis (Mann-Whitney U and Kruskal-Wallis tests) were used. Correlation

coefficient analysis was used to determine the association of one parameter versus another parameter of aqueous humour dynamics. $P < 0.05$ was considered statistically significant (all analyses, SPSS 24.0; SPSS, Chicago, IL).

Sample size calculation

The sample size estimate (30 patients) is based on the results of paired measurements of the three parameters in a previous study done at the Mayo Clinic, Rochester, MN, USA, by the KSL (Lim et al., 2008). Using these estimates, and the adjusted alpha (0.0167), detectable differences for each of the -3 primary parameters of interest were calculated, and the results are given in the following table:

Variable	Coefficient of Variation	Detectable Difference	Standard deviation	Power
IOP	20%	5%	2.2	90%
Aqueous flow	25%	5.4%	0.8	90%
Facility of outflow	37%	7.5%	0.1	90%

(Alpha=0.0167, two-sided t test of paired samples, 30 subjects)

Results

36 patients were invited to take part in this study. Three patients declined to participate, and another patient did not attend for their screening visit. One patient who had very high IOP after washout during safety visit was withdrawn from the study and had to have an urgent filtration surgery. One patient had satisfactory IOP after washout was also excluded from study.

Thirty eyes of 30 patients were included in the study. The study population comprised of predominantly African/Caribbean (n=18, 60%) and male (n=16, 53%) patients. The baseline characteristics are shown in Table 5-9. Primary open-angle glaucoma was the main diagnosis. At the baseline study visit, the mean wash-out IOP was 31.7±5.3 mmHg with an average number of medications of 3.2±0.7 (median of 3).

Thirteen fellow eyes of the recruited patients which did not receive any surgical intervention during or prior to the study formed the control group.

Table 5-9. Baseline characteristics of treated and control eyes are shown here

Mean±SD	Treated eyes (n=30)	Control eyes (n=13)	P value	95% CI
Age, years	60.1±13	60.3±13	0.7	-8.7 – 9.1
Ethnicity (African/Caribbean: White)	18: 12	7: 6	--	--
Diagnosis (OHT: POAG: PDG)	3: 26: 1	3: 10: 0	--	--
Gender (F: M)	14: 16	7: 6	--	--
BCVA	54.1±5.1	56.5±3.2	0.09	-5.3 – 0.4
CCT, µm	529±38	547±39	0.6	-8.5-44.0
ACD, mm	3.27±0.3	3.36±0.4	0.4	-0.1- 0.3
AXL, mm	24.1±1.1	24.1±0.9	0.7	-0.8- 0.7
WTW, mm	11.9±0.3	11.8±0.32	0.9	-0.3- 0.1
Number of meds	3.2±0.7 (median=3)	2.8±1.0 (median=3)	0.1	-0.1-0.9
MD, dB (mean±SD), range, median	-11.4±6.8 (-25-0.79), --10.3 (median)	-3.7±5.9 (-20-1.18), --2.61 (median)	**<0.001***	-11.7- -3.6

OHT: ocular hypertension, POAG: primary open angle glaucoma, PDG: pigment dispersion glaucoma, CCT: central corneal thickness, ACD: anterior chamber depth, AXL: axial length, WTW: white-to-white, MD: Mean deviation,

*statistical significance

The baseline aqueous humour dynamic measurements are shown in the Table 5-10. There was no statistically significant difference in all the parameters between the treated and controlled group.

Table 5-10. Baseline aqueous humour dynamics of the two groups

Mean±SD	Treated eyes (n=30)	Control eyes (n=13)	P value	95% CI
IOP (mmHg)	31.7±5.3	28.2±5.1	0.9	-0.7- 0.07
Aqueous flow rate (µl/min)	2.08±0.7	2.06±0.4	0.5	-0.6-0.3
TOF (µl/min/mmHg)	0.14±0.09	0.12±0.09	0.6	-0.08-0.04
Uveoscleral outflow (µl/min)	-0.87±2.17	-0.14±1.5	0.4	-0.5-2.2

HiFU treatment

HiFU treatment was performed in all 30 eyes. In all cases 6 sectors were treated except one patient who treatment was prematurely terminated by the operator whilst only 4 sectors were treated due to discomfort felt by the patient. Four patients experienced discomfort during the procedure, but it was perceived as mild to moderate and it did not cause interruption in treatment delivery.

Five subjects were considered as treatment failure (despite maximal medical therapy post HiFU treatment IOP remained suboptimal and they had to have glaucoma filtration surgery after HiFU application). A summary of treatment failure cases is shown in Table 5-11.

Table 5-11. Summary of treatment failure cases

cases	Diagnosis	Age (years)	Race	Number of Pre-op meds (no acetazolamide)	Pre-washout IOP*	post washout IOP*	Post op IOP* at 1 week	Number of meds at 1 week	Postop IOP* at 1 months	Number of meds at 1 month
1	PDG	40	White	4	28	40	33	2	46	3+ acetazolamide
2	POAG	72	White	2	26	37	13	0	29	4+ acetazolamide
3	POAG	74	African/Caribbean	4	23	33	14	0	27	4 +acetazolamide
4	POAG	45	African/Caribbean	3	25	30	10	0	45	3 +acetazolamide
5	POAG	53	African/Caribbean	4	24	37	22	0	28	3

PDG: pigment dispersion glaucoma, POAG: primary open angle glaucoma. *IOP was measured with a Goldmann applanation tonometer

Twenty-four patients completed 3-month aqueous humour dynamics measurements. One patient was lost to follow up and five patients had early treatment failure before 3 months wash-out and needed glaucoma filtration surgeries, and therefore withdrew from the study.

At 3-month post-operative visit (only cases who did not need any further glaucoma surgical interventions were considered), the mean post washout IOP was reduced by 16% (31.7±5.3 vs 26.6±4.8 mmHg, p=0.004) whilst aqueous flow rate was decreased by 15% (2.07±0.73 vs 1.77±0.55 µl/min, p=0.05) from baseline. Neither the tonographic outflow facility nor the uveoscleral outflow showed significant change from baseline. Table 5-12. illustrates primary outcome measures in the treatment group. The aqueous humour parameters remain unchanged at three months' in the control group as shown in Table 5-13.

Table 5-12. Aqueous humour parameters comparison before and after HiFU treatment

Mean±SD	Baseline (n=30)	3 months post operatively (n=24)	P value	95% CI	Effect estimate
IOP (mmHg)	31.7±5.3	26.6±4.8	**0.004**	1.3-6.4	30.5
Aqueous flow rate (µl/min)	2.07±0.73	1.77±0.55	**0.05§**	0.1-0.6	2.07
Tonography outflow facility (µl/min/mmHg)	0.14±0.09	0.15±0.13	0.6	-0.07- 0.05	0.14
Uveoscleral outflow (µl/min)	-0.79±1.75	-0.53±1.76	0.6	-1.2-0.7	-0.78

§ Wilcoxson signed ranked test, Bold font: statistically significant

Table 5-13. Aqueous humour parameters of the control group

Mean±SD	Baseline (n=13)	3 months post operatively (n=13)	P value	95% CI
IOP (mmHg)	28.2±5.1	26.8±3.4	0.2	-0.7-3.5
Aqueous flow rate (µl/min)	2.26±0.69	2.22±0.70	0.4	-0.5-0.2
Tonography outflow facility (µl/min/mmHg)	0.13±0.08	0.13±0.08	0.3	-0.05- 0.03
Uveoscleral outflow (µl/min)	-0.16±1.57	0.1±1.57	0.3	-0.84-0.3

Taking into account those five treatment failure eyes who were unable to undergo the 3 month's washout visit, proportion of eyes achieving IOP reduction of >20% at 3 months' wash-out visit compared to the pre-treatment wash-out IOP was 26.7% (n=8); for IOP reduction of >30%, the proportion of eyes was 10% (n=3). Table 5-14 summarises the peri- and post-operative complications.

Table 5-14. shows the list of adverse events § led to incomplete treatment

Lens opacity	**1**
Scleral marks	**4**
Pain during the procedure§	**4**
Persistent uveitis	**1**
Punctate epithelial erosions	**2**
Hypotony	**0**
Visual loss (> 2 Snellen lines)	**1**

Discussion

This is the first study investigating aqueous humour dynamics effect of a cyclodestructive procedure and specifically HiFU, in patients with uncontrolled open angle glaucoma on maximum tolerated medical therapy, and this is also the first washout study involving a cyclodestructive procedure. We demonstrated that there is a 20% risk of treatment failure (those who needed further glaucoma surgery intervention) within one month after a single HiFU treatment. Only 25 patients (80%) were able to undergo post-treatment washout measurements and in these eyes; HiFU reduced IOP 3-month postoperatively by 16% whilst aqueous flow decreased by 15%. without any significant effect on tonographic outflow facility and uveoscleral outflow. Only 26.6% of eyes achieved >20% IOP reduction at 3 months compared to baseline.

Ciliary body treatment for glaucoma has been available for over a century. The most obvious mechanism of action is the destruction of ciliary epithelium and subsequent reduction in aqueous humour production. Other possible adjunct mechanisms of action have been proposed such as increased uveoscleral outflow (Coleman et al., 1997) (external diode, Micro-pulse and HiFU) and flow through sclera (HiFU) (Mastropasqua et al., 2016). Although various indirect methods of measurements have strengthened this belief (Mastropasqua et al., 2016), there has never been any human aqueous humour dynamics study on the effect of ciliary body treatment.

Previous animal studies using HiFU in 18 rabbits' eyes, reduced IOP by over 50% four weeks after treatment (Aptel et al., 2010), while other clinical studies in human achieved between 25.5% and 38% of IOP reduction (F Aptel et al., 2016; Melamed et al., 2015; Aptel and Cyril, 2015; Florent Aptel et al., 2016). However, none of these clinical studies performed wash-out from glaucoma medications prior to study measurements. The reason for the discrepancies in the extent of IOP reduction between previous studies and our results may well be due to better glaucoma treatment compliance post treatment and heterogeneity of glaucoma diagnoses in previous studies. We only included open angle glaucoma cases whilst others included secondary glaucoma cases with variable degrees of response to HiFU therapy. Additionally, we reviewed patients more frequently post HiFU and encouraged compliance.

The most widely held view regarding the mechanism of IOP lowering effect post HiFU is through decreased aqueous production, which is consistent with our findings. However, there has been some speculations as to whether HiFU may affect uveoscleral outflow as well as other aqueous humour dynamics parameters (Mastropasqua et al., 2016). Mastropasqua et-al. (Mastropasqua et al., 2016) demonstrated anatomical alterations of sclera and conjunctiva four weeks after application of HiFU. They used these findings to contemplate that uveoscleral outflow enhancement may play a role in IOP reduction. However, the results from our aqueous humour dynamics measurement do not support this hypothesis. In fact, we did not find any statistically significant change in

uveoscleral and trabecular outflow 3 months' after the HiFU treatment. Although earlier effects on other aqueous humour dynamics parameters such as uveoscleral outflow before our three months' measurement cannot be ruled out by our study.

In the aforementioned histological study of rabbits' eyes which had underwent HiFU treatment, the authors (Aptel et al., 2010) described coagulation necrosis lesions in the ciliary processes post HiFU treatment. They observed the lesions were circumferentially distributed with the loss of the bi-stratified epithelium, and oedema and vascular congestion of the ciliary stroma without any histological changes to the scleral tissue adjacent to the treated area. These findings appeared to support the aqueous humour dynamics findings from our study of reduced aqueous flow rate.

In order to explore if the aqueous flow rate change can fully account for the reduction in IOP at three months', we made some calculations using the Goldmann's equation (see Appendix 1); If we assume that other aspects of aqueous dynamic parameters, such as tonographic outflow facility (C), uveoscleral outflow (*Fu*) or episcleral venous pressure (*Pe*) are not affected by HiFU and the mechanism of IOP lowering effect of HiFU is solely via the decreased aqueous flow rate (Ff) alone, then according to Goldmann's equation, a 16% reduction in intraocular pressure (Pi) at 3-month seen in our study should correspond to 15% decrease in aqueous flow rate (Ff) and this correlated well with our measured reduction of 12% in aqueous flow rate at 3 month. As this

largely accounts for the extent of IOP reduction, one can therefore conclude that the reason for the IOP drop post HiFU is likely to be caused by a decrease in aqueous flow rate alone (see Appendix 1).

The current study has few limitations; firstly, we did not perform any aqueous humour dynamics study measurement prior to 3 months visit and there may have been changes in the other aspects of aqueous humour dynamics parameter, such as uveoscleral outflow then; however, aqueous flow measurement using fluorophotometry can be inaccurate in active uveitis. This is due to the presence of excessive protein in the anterior chamber and the breakdown of the blood aqueous barrier (during early postop period after HiFU) can distort the assumptions about the standard diffusional loss of fluorescein during fluorophotometry (Brubaker and McLaren, 1985). Furthermore, the use of topical steroid may potentially adversely affect the trabecular outflow (Overby et al., 2014; Johnson et al., 1997). We have therefore chose the three months' timepoint as the first opportunity, after the cessation of topical steroid use and post-operative uveitis resolved to perform aqueous humour dynamics measurement. Secondly, none of our patients had repeat HiFU treatment. This is in contrast to all previous studies which patients had more than one application of HiFU treatment. Repeated treatment application may augment the effect of HiFU. However, as our primary interest was in the aqueous humour dynamics effects of HiFU treatment, repeated treatment would have caused further intraocular inflammation, thus delaying the aqueous humour dynamics

measurement further in some patients. The result of our study may not be generalizable to other types of cyclodestructive treatments such as cyclodiode as there may be a different mechanism of action due to laser light scatter and potentially wider treatment area.

In conclusion, our study is the first clinical study to show aqueous humour dynamic changes after HiFU treatment. We confirmed that a three months' post-HiFU treatment, reduction in aqueous production is the only aqueous dynamic parameters that could have accounted for the drop in IOP.

Appendix 1. Goldmann equation and calculations

The aqueous production (flow) rate is equal to the sum of trabecular outflow and uveoscleral outflow

$$Ff = (Pi - Pe).C + Fu$$

where, Ff is aqueous humour flow measured by fluorophotometry, Pi is intraocular pressure in the anterior chamber, Pe is the episcleral venous pressure, C is trabecular outflow facility measured by tonography and Fu is uveoscleral outflow.

In this study, "*Ff*" was measured at baseline and at 3 months, while HiFU is assumed to have no effect on *Fu, C* and *Pe*. We considered *C* to be 0.2-µl/min/mmHg, *Fu* to be 1 µl/min and *Pe* to be 8 mmHg. Therefore, a 16% drop in *Pi*, consistent with the IOP decrease observed at 3 months, should correspond to 12% reduction in "*Ff"*.

Chapter 6|Discussion and Conclusions

Discussion

Within this thesis, I have explored the aqueous humour dynamics changes in uveitis and the effect of phacoemulsification and high intensity focused ultrasound.

Within the discussion, research findings have been considered and related manuscripts have been included. However, in this chapter, a summary will be given to draw a conclusion on each finding of each individual project.

Limitations will be discussed before reviewing the implications of the research and considering what direction the research and the field should go in the future. Each section below will give a brief summary of the different areas that this research has been based upon. When all information is gathered and culminated, then the findings can be discussed against previous and current research.

The effect of phacoemulsification on outflow facility

There has been an explosion of new glaucoma procedures combined with cataract surgery called minimally invasive glaucoma surgery (MIGS). However, the effect of modern phacoemulsification on IOP has not been emphasised enough. In previous studies, it has been shown that IOP reduced after cataract surgery on average by 1.5-9 mmHg (this varies depending on the starting IOP prior to cataract surgery) (Slabaugh et al., 2014; Mansberger et al., 2012; Pfeiffer -et al., 2015). The higher the baseline IOP, the greater the IOP reduction. In a more recent study in Hydrus II trial, IOP was reduced in the phaco alone group by 9-mmHg (Pfeiffer et al., 2015). The cases had higher baseline IOP with consequently greater IOP reduction postoperatively. In my study, however, IOP was reduced by 2 mmHg only. Perhaps regression to mean phenomenon may be an explanation to this difference between studies (Pfeiffer et al., 2015). There have been several speculations about the mechanism of action for IOP reduction in cataract surgery especially in angle closure but not so much in open angle glaucoma (OAG). It has been proposed that chemokines and inflammatory molecules play important roles to improve IOP (Wang et al., 2003; Tsuboi et al., 2012). Another hypothesis is the ultrastructural changes as a result of vibration of TM from phacoemulsification (Mehdizadeh, 2008; Zhao et al., 2016). All these suggest an improvement in the trabecular outflow facility.

In my study, I have demonstrated that IOP decreased by 12% after cataract surgery and it was maintained during the course of the study for a year. At the same time the TOF enhanced by 15% which corresponded with the amount of IOP reduction; that is with the assumption that EVP and aqueous flow rate remained unchanged after cataract surgery. This finding highlights the importance of the cataract surgery alone, in the management of OAG/OHT cases as a sole therapy. Further enhancement of IOP could be imagined if cataract surgery is combined with other procedures as it has been shown in the literature (Pfeiffer et al., 2015; Samuelson et al., 2011).

Aqueous humour dynamics in uveitic eyes

Uveitis is potentially a blinding condition. This is mostly due to the development of uveitis sequlae such as glaucoma. The evaluation of uveitis influence on aqueous humour dynamics (ADS) changes can be challenging. This is due to active inflammation which can hamper the current techniques of measuring aqueous flow rate i.e. fluorophotometry (Brubaker and McLaren, 1985). This method relies on the assumptions that the amount of fluorescein leakage through aqueous-blood barrier (ABB) is minimal/negligible. However, it is known that the ABB could be compromised in active uveitis (Toris and Pederson, 1987). The effect of uveitis on ADS has been under explored due to the above-mentioned reasons. To date there has been one animal study in monkey eyes to assess the ADS changes in uveitis. Toris and Pederson (Toris and Pederson, 1987), developed an experimental animal model of uveitis by injecting albumin in the monkey eyes. They showed that in the active phase the aqueous flow rate and trabecular outflow facility are decreased whilst the uveoscleral outflow is increased. Consequently, IOP was reduced, with some cases of hypotony. However, evaluating the ADS with non-invasive methods in humans in acute stages of uveitis has been proven to be challenging. To date there has been only two studies in humans. Johnston et al. (Johnson et al., 1983) performed ADS in Fuch's-heterochromic cyclitis cases. They compared the ADS in the affected eyes with contralateral healthy eyes. They showed that the

aqueous flow rate (AF) remained unchanged. But the issue is that the ABB is not intact in FHC due to persistent anterior chamber flare/cells. Therefore, any attempt in measuring AF based on fluorescein clearance may not be accurate. In another study Ladas et al. (Ladas et al., 2001) performed flare meter and Schiøtz-tonography in uveitis eyes. They showed that the higher the flare, the lower the outflow. But in that study the AF was not measured.

In my project three groups of patients were utilised:

Group1: Uveitis with glaucoma/OHT

Group 2: Uveitis with normal IOP

Group 3: Healthy volunteers

This was a cross-sectional study to investigate the effect of uveitis on aqueous humour parameters. All patients were clinically inactive at the time of the study measurements. They had wash-out of their glaucoma and steroid medications (in group 1). I demonstrated that in group-1, TOF was decreased whilst IOP was increased. However, uveoscleral outflow and aqueous flow rate remained unchanged across all three groups. Therefore, I proposed that the aqueous shut down after glaucoma operation in uveitic eyes might be inaccurate. However, this claim maybe challenging to substantiate and might be controversial. Consequently, a good filtration surgery would be sufficient to control IOP in patients with mild to moderate severity of recurrent uveitis. One interesting finding from my study was that nearly half of the patients (47%) in group 1, after the washout of their anti-hypertensive medications maintained normal IOP.

One recommendation from this would be to perform a trial of glaucoma medications washout after the subsidence of the acute phase of uveitis. However, those patients will still need close monitoring to ensure that any future IOP spike as a result of uveitis reactivation is treated swiftly and in timely manner. This will in turn reduce the burden of the disease financially and emotionally.

The effect of high intensity focused ultrasound on aqueous humour dynamics

High intensity focused ultrasound (HiFU) has been introduced into the clinical practice since the 1940's. It was first used to treat Parkinson disease (Lynn and Putnam, 1944). Over the years, application of this modality has become broadened. HiFU was first utilised in ophthalmology by Jackson Coleman and colleagues in the 1980s (Lizzi et al., 1984; Coleman et al., 1986). They applied HiFU in a variety of pathologies including ocular oncology, retinal detachment and in glaucoma. The initial result of using Sonocare was very promising. However, the glories of the treatment were soon diminished due to a high rate of complication, cumbersome technique with a large device and inconsistent outcomes (Maskin et al., 1989; Sterk et al., 1992). HiFU was soon abandoned in the early 1990s. But in 2011 the EyeTech company revisited the idea of utilising HiFU in the glaucoma treatment (Charrel et al., 2011). The new device which is called EyeOP 1 was miniaturised and simplified. With the new and improved model, its success was more promising in early animal and human trials in the refractory glaucoma cases in blind eyes (Melamed et al., 2015; Aptel and Lafon, 2012; Aptel et al., 2014). This was then led to larger clinical trials to confirm the effectiveness of this treatment (F Aptel et al., 2016). However, the mechanism of action has not been fully understood. There has been only one study facilitating anterior segment OCT to assess the scleral

changes as a proxy for uveoscleral outflow alterations following HiFU. Mastropasqua et al. (Mastropasqua et al., 2016) demonstrated that one-month post HiFU treatment the number of the sizes of the scleral lakes increased and they used this as a proxy for uveoscleral outflow enhancements.

The impact of HiFU on aqueous humour dynamics parameters has not been studied and the mechanism of action for HiFU has been broadly hypothesized. In my project the patients that were selected had OAG whose IOP was inadequately controlled; they were also due to have glaucoma filtration surgery. Patients had a washout from their hypotensive medications 4 weeks prior to their study visit, this was prior to the HiFU application.

All patients had fluorophotometry and Schiøtz tonography. These measurements were repeated 3 months after treatment (patients had wash-out from their anti-glaucoma meds for 4 weeks prior to the study visit). At 3 months postoperatively, IOP was reduced by 16% and aqueous flow rate was reduced by 15%. However, there were no detectable changes in uveoscleral outflow and tonographic outflow facility. The level of IOP reduction was slightly lower than previous reports. One reason might have been due to the fact that the patients were only allowed to have one application of HiFU. This is in contrast to previous studies that patients had multiple applications of HiFU. Additionally, patients in this study had an anti-glaucoma medication washout prior to their study visit. The complete success in my study was (IOP reduction >20%) only-27%. I demonstrated that the calculated uveoscleral outflow remained

unchanged 3-month post treatment. Mastropasqua et al. (Mastropasqua et al., 2016) showed some scleral changes 1 months after the HiFU treatment. They suggested that their findings might be a surrogate of enhanced uveoscleral outflow. One reason for this discrepancy of findings might be due to the fact that the early scleral alterations are transient effects of HiFU. Nonetheless, longer-term studies will be required.

Contributions to the literature and implications

The work in this thesis contributes significantly to what is known about the effect of phacoemulsification, HiFU and uveitis on the aqueous humour dynamics parameters. None of the previous studies have been as comprehensive (with the available technology). These studies further highlighted the pathophysiology of IOP changes in the aforementioned scenarios.

Limitations

Important limitations to this work must be acknowledged and have already been discussed in each chapter. The inherent flaws of the current aqueous humour dynamics measurements in humans have been discussed in detail in chapter 2. The Schiøtz tonography has its own limitations including several assumptions which govern the accuracy of the device. The accurate measurement of uveoscleral outflow and episcleral venous pressure is not currently possible. In this thesis the results have been based upon the assumed episcleral venous-pressure; that has been used according to measurements extracted from previous studies. The calculated uveoscleral outflow was worked out through the Goldmann formula.

Future directions

Following the work of this thesis, there are a number of areas in which it would be interesting to investigate.

Further collaborative work would lead to the development of more accurate and direct methods which are non-invasive to be carried out in humans. As it has been shown by the previous partnership in the development of the new digital Schiøtz tonography machine. It would be useful to conduct some non-invasive animal studies based on cannulations and direct methods.

Secondly, uveitic glaucoma is a fascinating area that could be investigated further, particularly the ADS changes during the acute phase and in more severe cases of uveitis. However, the challenge is that acute inflammation limits the application of current techniques in measuring the aqueous humour dynamics. So, a technique which is direct and non-invasive would be ideal for active phase of inflammation. Another aspect to explore in the uveitic glaucoma is to consider performing laser flaremetry for clinically quiescent uveitis to establish subclinical activity of the disease. Additionally, it is crucial to monitor uveitis case who are on glaucoma medication very closely. This is to capture early IOP spike in cases of uveitis flare-up.

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Appendix

Data collection sheet for the effect of phacoemulsification on the facility of outflow

Aqueous Humor Dynamics in Patients with Glaucoma or Ocular hypertension: An Observational Study

Patient Data Sheet(Follow up visit)

Patient's sticker

Study Number: ***Study eye:*** *Right/Left*

Date of surgery :

Follow up visit: Day 1, Week 1, Month 1, Month 3, Month 6, Month 12 *(please circle one)*

Surgical Complications:

	Right eye		Left eye	
BCVA +/- pinhole snellen				
BCVA +/- pinhole LogMar				
IOP (Goldmann)				
AC activity				
Disc				
Humphrey visual fields				

Has the fluorophotometry and tonography performed today?

Fluorophotometry		
Time +/- 1 hour	Time	
10am		
11am		
12pm		
1pm		
Aqueous flow rate	RE:	LE:

Refraction				
Keratometry	K1	K2	K1	K2

Time	RE icare	RE pneumo	LE iCare	RE penumo
10.10am				
11.10am				
12.10am				
13.10am				

facility of outflow	RE:	LE:

Tonographic outflow facility changes

Patient Data Sheet (Follow-up)

Name:	
DOB:	Age:
Hospital Number:	
Male / Female:	

Study Number: ***Date of surgery***: *Right eye / Left eye*

Surgical Complications:

Time: ***Power:***

Follow up	1 Day	1 Week	1 Months	3 Months	6 Months
Date					
Diagnosis					
Current ocular treatment:					
BCVA ± pinhole acuity					
Anterior segment					
Gonioscopy (Shaffer's, PAS)					
IOP					
AC activity					
Disc					
Visual Field					
T.O.F					

Data collection sheets for aqueous humour dynamics in uveitic eyes

The contribution of altered aqueous dynamics to the development of raised intraocular pressure in patients with uveitis

Date: → Patient name: ····· → → → **PI: Mr Sheng Lim ext no 84885**

Phone no:	DOB:	Age:
UVEITIC RAISED IOP	UVEITIC NORMAL IOP	CONTROL
Study no:	Hosp no:	M/F:
PMHx:	Other eye disease	
Type of uveitis: LE: RE:	Allergies:	
Regular eye drops:	Eye drops post washout:	Systemic meds:
LASIK: Y / N	Prev eye surg: Y / N	Cannabis use: Y / N
Ethnicity: White Asian Black Chinese	FHx glaucoma? Y / N	
Total number of uveitis attacks: RE: ····· LE:		
Date of resolution of most recent attack: RE: ····· LE:		
Previous highest IOP: RE: ····· LE:		

Number of uveitis attacks

Eye	Date	Severity* (cells) 0 – 4+	Severity** (flare) 0 – 4+	IOP	Duration	Treatment

Patient name: → → → DOB: ····· **PI: Mr Sheng Lim ext no 84885**

	Right eye	Left eye
BCVA +/- pinhole snellen		
BCVA +/- pinhole LogMar		
CCT		
AC depth and AL		
Gonioscopy		
IOP (Goldmann)		
AC activity		
Disc		
Humphrey visual fields		
Van Hericks		

Blood pressure 1:	Blood pressure 2:
Total F drops RE:	Total F drops LE:
Time of fluorescein insertion last night	

Fluorescence

Time +/- 1 hour	Time
10am	
11am	
12pm	
1pm	
Aqueous flow rate	RE: ····· LE:

Tonometry/IOP (icare and pneumo)

Time	RE icare	RE pneumo	LE icare	LE pneumo
10.10am				
11.10am				
12.10am				
13.10am				

Trabecular outflow: RE ····· LE	Time:

*

Grade	Cells in field
0	<1
0.5+	1-5
1+	6-15
2+	16-25
3+	26-50
4+	>50

**

Grade	Description
0	Nil
1+	Faint
2+	Moderate (iris and lens details clear)
3+	Marked (iris and lens details hazy)
4+	Intense (fibrinous exudate)

Data collection sheets for the effect of high intensity focused ultrasound on aqueous humour dynamics. All participants denied cannabis consumption.

The Effect of High Intensity Focused Ultrasound (HiFu) on Aqueous Humor Dynamics in Patients with Glaucoma or Ocular Hypertension: An Observational Study

Patient Data Sheet (Baseline)

Date:

Patient's sticker

Study Number:

Race White Asian Black Chinese

Consented for the trial(version...)? Cannabis use: Yes / No (please circle)

Fluoresceine drops used last night Beta blocker use: Yes / No (please circle)

Glaucoma drops washed out

Study Measurements:

Diagnosis	RE	LE
Other ophthalmic problems:		
Ocular treatment prior to wash out:		
BCVA ± pinhole acuity (LogMAR)		
BCVA ± pinhole acuity (Snellen)		
CCT		
AC depth & AL		
Gonioscopy		
IOP		
AC activity		
Disc		
Humphrey Visual Fields (MD, PSD)		
T.O.F		
Aqueous flow rate		

Tonometry/IOP (icare and pneumo)

Time	RE icare	RE pneumo	LE icare	LE pneumo
9.10am				
10.10am				
11.10am				
12.10am				

The Effect of High Intensity Focused Ultrasound (HiFu) on Aqueous Dynamics in Patients with Glaucoma or Ocular Hypertension: An Observational Study

Patient Data Sheet (Follow-up)

Patient's sticker

Study Number: ***Date of surgery*** : ***HiFU*** *Right eye / Left eye*

Surgical Complications:

Time:		***Duration:***		***Sectors treated:***	

Follow up	1 Day	1 Week	3 Months	6 Months	12 Months
Date					
Diagnosis					
Current ocular treatment:					
BCVA ± pinhole acuity					
Anterior segment assesment					
Gonioscopy (Shaeffer's, PAS)					
IOP					
AC activity					
Disc					
Visual Field					
T.O.F					
Aqueous flow rate					

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Effect of phacoemulsification on facility of outflow

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ABSTRACT

Purpose Phacoemulsification has been shown to reduce intraocular pressure (IOP). The mechanism of action is thought to be via increased trabecular outflow facility. However, studies on the relationship between phacoemulsification and outflow facility have been inconsistent. This study intended to examine the change in electronic Schiotz tonographic outflow facility (TOF) and IOP measurements following phacoemulsification.

Methods Patients who were due to undergo a standard clear corneal incision phacoemulsification with intraocular lens (IOL) implantation, at St Thomas' Hospital, were invited to participate in this study. IOP was measured using Goldmann's applanation tonometer, and TOF was measured by electronic Schiotz tonography at baseline and at 3, 6 and 12 months postoperatively.

Results Forty-one patients were recruited. Tonography data for 27 patients were reliable and available at all time points. Eleven cases had primary open angle glaucoma and cataract, while 16 patients had cataract only. Mean IOP reduced at every time point postoperatively significantly compared with baseline. TOF improved significantly after cataract extraction at all time points (baseline of 0.14±0.06 vs 0.18±0.09 at 3 months, P=0.02 and 0.20±0.09 at 6 months, P=0.003, 0.17±0.07 µL/min mmHg at 12 months, P=0.04). Five contralateral eyes of patients with cataracts only who did not have any intraocular surgery during the follow-up period were used as comparison. Their IOP and TOF did not change significantly at any postoperative visits.

Conclusion This is the first study using electronic Schiotz tonography with documented anterior chamber depth and gonioscopy after modern cataract surgery (CS) with phacoemulsification and IOL implantation. We demonstrated that phacoemulsification increases TOF and this fully accounts for the IOP reduction following CS.

ISTCRN registration number ISRCTN04247738.

INTRODUCTION

Several studies explored the effects of phacoemulsification on intraocular pressure (IOP) changes at short (6 months), medium (36 months) and long term (60 months).[1–5] The reported IOP reduction varies between 1.5 and 9.0 mm Hg.[5–7] Different mechanisms of action have been proposed for the IOP-lowering effects following cataract surgery (CS), including the mechanical influence of the lens removal,[6] increased uveoscleral outflow and increased trabecular outflow.[8] However, there are no studies using electronic Schiotz tonography assessing the effect of modern small incision phacoemulsification with intraocular lens (IOL) implantation on trabecular outflow facility.

Lee and Trotter[9] investigated the effect of extracapsular cataract extraction without IOL implantation on the facility of outflow. They used electronic Schiotz tonography in patients with cataract of whom seven cases had open or closed angle glaucoma and 11 cases of pseudoexfoliation. They showed that facility of outflow decreased within first 3 weeks postoperatively but then returned to preoperative values within 4 months after the operation. However, in this study, outflow facility changes were very variable. Additionally, they had included mixed cases of complicated surgeries such as vitreous loss and the follow-up was only 6 months.

Another study by Meyer and associates,[10] demonstrated that pneumatonographic outflow facility after phacoemulsification improved on the first day after surgery; however, outflow facility at 1 year (0.46±0.38 µL/min mm Hg) was not statistically different compared with the baseline of 0.39±0.23 µL/min mm Hg.

To date, there have been no studies using electronic Schiotz tonography (which is a method of outflow facility measurement with less intersubject and interobserver variability compared with pneumatonography[11–13]) to determine the effect of modern small incision phacoemulsification CS on tonographic facility of outflow.

METHODS

This research conformed to the tenets of the Declaration of Helsinki. Patients with visually significant cataract with or without primary open angle glaucoma (POAG), who were due to undergo phacoemulsification with IOL implantation, were enrolled in this prospective study. The recruitment took place between September 2009 and May 2011. POAG was defined as IOP >21 mmHg on at least one occasion and abnormal visual field testing with corresponding optic disc changes. Only one eye per patient was included in the final analysis. When both eyes were eligible, only the first eye to be operated on was included in the analysis. Patients were provided with study information at the initial contact, and signed informed consent was sought before measurements and the surgery. Patients with any history of intraocular or keratorefractive surgery, any secondary glaucoma including traumatic, neovascular, uveitic, pseudoexfoliative and pigment dispersion syndrome were excluded.

Contralateral eyes of the study patients, which did not undergo any intraocular surgery during the follow-up period, with available tonographic

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outflow facility (TOF) and IOP data at all time points postoperatively, were used for the comparison purposes.

Measurements

All patients underwent a comprehensive ophthalmic examination before the operation, including visual acuity measurement (LogMAR), slit lamp biomicroscopy, gonioscopy, IOP measurement using Goldmann's applanation tonometer, anterior chamber depth (ACD) and axial length (AXL) measurement using IOL Master (Carl Zeiss Meditec, Dublin, California, USA), central corneal thickness (CCT; Pachmate DGH 55, DGH Technology, Exton, Pennsylvania, USA), static automated visual field (Humphrey automated white-on white, 24–2 SITA-standard; Carl Zeiss Meditec), and dilated ophthalmoscopic examination.

TOF (TOF='C') was measured with an electronic Schiotz tonographer (model 720; Berkeley Bioengineering, San Leandro, California, USA) on the day of the surgery (between 09:00 and 11:00) prior to the operation and then repeated at 3, 6 and 12 months postoperatively. The facility of outflow was measured from the rate of the decay of IOP in the supine position during application of a recording Schiotz tonometer probe on the cornea, over a period of 4 min with a standard 5.5 g weight.[12] Nine readings at 30 s intervals were manually entered the McLaren tonography computer program.[11] The programme fits a second-degree polynomial by the method of least squares to nine data points and determines by extrapolation, the best-fit values at time 0 and 4 min. The values at 0 and 4 min are then used to calculate 'C' based on standard nomograms.[12 14]

Data collection and outcome measures

Data including age, gender, race, IOP, TOF, CCT, AXL, anterior chamber depth, gonioscopy, vertical cup: disc ratio and mean deviation in visual field testing were recorded.

Primary outcome measures were TOF (C) and IOP at 3, 6 and 12 months' postcataract extraction.

Surgical procedure

CS was performed by an experienced surgeon (KSL) under local or general anaesthesia. A clear corneal incision (2.8 mm) was made and followed by a paracentesis and the injection of viscoelastic. Then capsulorrhexis and hydrodissection was performed. The lens was removed by phacoemulsification of the lens nucleus and aspiration of the cortical lens matter. After further injection of the viscoelastic, an acrylic injectable IOL (AcrySof SA60AT, Alcon, Texas, USA) of the appropriate power was inserted into the capsular bag. The viscoelastic was washed out and an intracameral antibiotic injection was given at the end. Patients received Maxitrol (Neomycin sulfate 3500 IU/mL, Polymyxin B sulfate 6000 IU/mL and 1 mg dexamethasone, Alcon Laboratories, UK) four times daily for 2 weeks and then twice daily for another 2 weeks postoperatively. All patients were off steroid treatment after this period.

Sample size calculation

The SD of outflow facility was derived from a previous study by one of the authors.[11] This study had a 90% chance of finding an 8.4% difference in outflow facility and 5% difference in IOP among two groups, if these differences existed (n=25 subjects, α=0.05 and β=0.10). There was no difference between the two groups in terms of relative changes in facility; therefore, we used combined data into one group of 25.

Statistical analysis

Student's t-test and one-way analysis of variance (ANOVA) for repeated measures were used to compare continuous variables among groups. The 95% CIs for the mean difference between pairs for each outcome measure were calculated. Linear regression analysis was used to determine the correlation between IOP, facility of outflow and differences from baseline. P<0.05 was considered to be significant (IBM SPSS V.23.0).

RESULTS

Forty-one patients were enrolled in the study. Eight patients withdrew after signing the consent, due to their time constraints and inability to attend all required postoperative visits. Three subjects had poor tonography tracings on at least one time point (10% rejection rate in aqueous humour dynamic measurement is similar to previous studies).[11] One case was excluded due to an intraoperative complication (posterior capsular rupture and vitreous loss) and two further cases were omitted due to persistent postoperative uveitis. In total, data from 27 patients with reliable tonographic outflow tracings at baseline and all subsequent study visits were included in the final analysis. Only one eye from each patient was used for the analysis.

Sixteen individuals had cataract only, while 11 cases had an existing diagnosis of POAG. The average age was comparable (in cataract cases mean age was 67±11.2 years, while in POAG group it was 73±7.2 years, P=0.09). Other baseline characteristics of each group is shown in table 1.

Baseline IOP was similar in each group (15.7±2.7 mm Hg in non-glaucomatous cases vs 16.3±4.8 mm Hg in POAG with cataract group, P=0.7, 95% CI −3.6 to 2.4).

Overall, IOP reduced by 2.0±3.2 mm Hg at 3 months (12% decrease), while at 6-month and 12-month visits, it only reduced by 1.7±3.4 (10% decrease) and 2.0±3.6 mm Hg (10% decrease), respectively (table 2 and figure 1). We used one-way ANOVA for repeated measures to compare IOP between each visit. The IOP reduction at all post-operative visits was statistically significant (3 months P=0.003, 6 months P=0.04 and 12 months P=0.02).

TOF improved significantly at all postoperative time points after cataract extraction compared with the baseline (table 3 and figure 2). However, TOF enhancement did not differ between each visit using one-way ANOVA for repeated measures (at 3 months P=0.7, at 6 months P=0.4 and at 12 months P=0.2). There was no statistically significant correlation between phaco power and TOF at any time point (at 3 months P=0.5, at 6 months P=0.4 and at 12 months P=0.7).

The average postoperative IOP in cataract and POAG cases are shown in table 2 and figure 3. Mean IOP in cataract cases at 12 months' postsurgery was 13.7±3.0 mm Hg; this was comparable to POAG cases in which IOP decreased to 14.2±3.7 mm Hg (P=0.7, 95% CI −3.0 to 2.2). Overall, baseline IOP was a moderate predictor of postoperative IOP reduction at all time points (P=0.004, R=0.53).

Additionally, there was no statistically significant correlation between TOF changes and IOP alterations at any time point (P=0.08, r^2=0.1) (figure 4). Furthermore, there was no statistically significant correlation between AXL and ACD with IOP and TOF (P=0.9, r^2=0.002).

The number of glaucoma medications and treatment regime remained unchanged in the POAG group at baseline and at all postoperative visits. All patients were on prostaglandin analogues. Additionally, five patients were taking beta blockers, four patients carbonic anhydrase inhibitor and one patient was on alpha-2 agonist medications. IOP and TOF measurements

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Table 1 Baseline characteristics

	Cataract only (n=16)	POAG and cataract (n=11)	P value	95% CI	Overall (n=27)
Age (years)†, range	67±11.2 (43–82)	73±7.2 (59–83)	0.09	−14.8 to 1.1	69±10.2 (43–83)
Gender (F:M)	9:7	3:8	0.4	–	12:15
BCVA	0.3±0.4	0.3±0.2	0.7	−0.2 to 0.3	0.3±0.3
Ethnicity (Asian:Black:White)	0:4:12	2:6:3	0.5	–	2:10:15
ACD (mm)†, range	3.25±0.36 (2.9–3.8)	3.22±0.48 (2.85–4.1)	0.8	−0.3 to 0.3	3.23±0.40 (2.85–4.1)
AXL (mm)†, range	23.3±0.8 (22.8–24.5)	23.9±0.94 (22.5–25.7)	0.06	−1.3 to 0.05	23.6±0.89 (22.5–25.7)
CCT (µm)†, range	535±27 (497–596)	543±27 (507–580)	0.5	−29.4 to 14.1	538±26 (497–596)
IOP (mm Hg), range	15.7±2.8 (10–22)	16.3±4.8 (10–26)	0.7	−3.50 to 2.4	15.9±3.66 (10–26)
TOF (µL/min/mm Hg), range	0.15±0.06 (0.06–0.29)	0.13±0.08 (0.04–0.29)	0.4	−0.03 to 0.08	0.14±0.06 (0.04–0.29)
HVF (mean deviation)‡, range	2.5±0.6 (1.0–3.0)	−11.7±1.9 (−14.0 to2.0)	<0.001*	–	−3.1±8.84 (−14 to 3)
Cup disc ratio‡	0.3 (0.2–0.7)	0.6 (0.6–0.9)	<0.001*	–	0.5 (0.2–0.9)
Phaco power, %, range	19.8±8.7 (10–31)	15.9±5.0 (10–27.9)	0.06	−2.1 to 9.9	18.2±7.5 (10–31)
Phaco time, minutes, range	2.25±4.12 (1.0–3.4)	1.54±0.54 (0.5–2.3)	0.16	−1.8 to 3.3	1.9±3.13 (0.5–3.4)

*Statistically significant.
†Student's t-test (two sided).
‡Mann-Whitney test.
ACD, anterior chamber depth; AXL, axial length; BCVA, best corrected visual acuity; CCT, central corneal thickness; HVF, Humphrey visual field; IOP, intraocular pressure; POAG, primary open angle glaucoma.

were not statistically different in any particular group of these patients (P=0.35).

The IOP reduction was statistically significant at all postoperative visits in cataract groups but not in the POAG group (table 2 and figure 3).

While TOF in cataract group only improved at 3 and 6 months postoperatively, in the glaucoma group only month 6 TOF enhancement was statistically significant.

The comparative data is presented in figure 5.

Overall, baseline TOF was weakly correlated to TOF at 3 month postoperatively (P=0.05, r=0.40) (figure 6).

We identified five patients (with cataract) who did not undergo any intraocular surgery in their contralateral eyes during the follow-up period and used their data as comparison. The average age of this group was 64.4±7.4 years. They all had open angles on gonioscopy (Shaffer's grading of 3 or 4 in all quadrants) with mean anterior chamber depth of 2.98±0.62 mm. The baseline IOP for this group was 18.0±2.09 mm Hg, which did not change significantly at 3, 6 and 12 months' visits (15.2±1.90 mm Hg at 3 months, P=0.84, 16.4±2.07 mm Hg, P=0.21 and 16.8±2.71 mm Hg at 12 months, P=0.29) (figure 7). Similarly, the facility of outflow at 3, 6 and 12 months compared with baseline was not statistically different at any time point (0.18±0.08 vs 0.18±0.09 at 3 months, P=0.1, 0.18±0.09 at 6 months, P=0.55 and 0.18±0.07 µL/min mm Hg at 12 months, P=0.6) (figure 8).

DISCUSSION

This is the first study to assess the facility of tonographic outflow after modern small incision phacoemulsification with IOL implantation using electronic Schiotz tonography. We demonstrated that IOP was significantly reduced at 3, 6 and 12 months after CS. In this study, the overall mean IOP reduction varied between 10% and 12%. There were also corresponding positive enhancement of TOF at all time points.

Numerous studies have explored the effect of phacoemulsification CS on IOP; all demonstrated significant but variable fall in IOP (1.5–9.0 mm Hg) postoperatively.[4 6 15–19] However, current evidence is very limited on the effect of phacoemulsification on the facility of outflow.

In the Ocular Hypertension Treatment Study (OHTS), the IOP reduction was 4.0 mm Hg, 3 years after CS,[5] which was greater than observed IOP reduction in our study at 12 months (2.0±3.6 mm Hg). However, in the OHTS, baseline IOP was slightly higher (19.8±3.2 mm Hg) compared with our cohort (16.0±3.7 mm Hg). The IOP reduction after phacoemulsification has been shown to be proportional to the preoperative IOP, with significantly greater IOP reduction observed among those

Table 2 This table illustrates IOP reduction at postoperative visits at 3, 6 and 12 months compared with baseline IOP

	IOP at baseline	IOP at 3 m (mm Hg), % of reduction	Comparison made to baseline	IOP at 6 m (mm Hg), % of reduction	Comparison made to baseline	IOP at 12 m (mm Hg), % of reduction	Comparison made to baseline
Cataract group (n=16)	15.7±2.8	13.4±2.7 (13)	P=0.009*, 95% CI 0.6 to 3.8	14.4±2.6 (7)	P=0.005*, 95% CI 0.17 to 2.6	13.7±2.9 (12)	P=0.006*, 95% CI 0.6 to 3.2
POAG group (n=11)	16.3±4.8	14.5±3.2 (6)	P=0.1, 95% CI −0.7 to 4.2	14.0±3.6 (9)	P=0.1, 95% CI −0.8 to 5.1	14.2±3.7 (8)	P=0.2, 95% CI −1.3 to 5.5
Overall (n=27)	15.9±3.66	13.9±2.9 (12)	P=0.003*, 95% CI 0.7 to 3.3	14.3±3.0 (10)	P=0.02*, 95% CI 0.3 to 2.9	13.9±3.2 (10)	P=0.008*, 95% CI 0.5 to 3.4

Additionally, percentage of IOP reduction is shown.
*Statistically significant.
IOP, intraocular pressure; POAG, primary open angle glaucoma.

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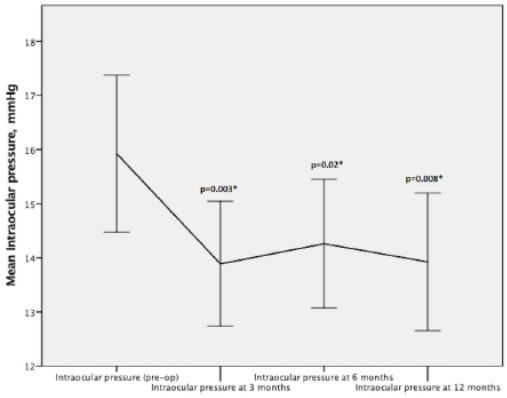

Figure 1 Overall intraocular pressure (IOP) changes on each time point postoperatively compared with baseline IOP. *Statistically significant.

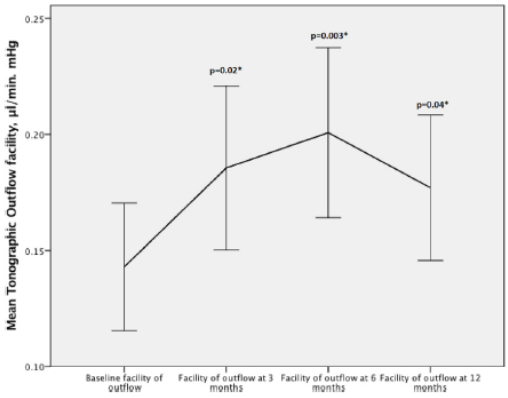

Figure 2 Overall tonographic outflow facility changes at each postoperative visit compared with baseline intraocular pressure. *Statistically significant.

with higher preoperative IOP[7]; this may explain the greater IOP reduction in the OHTS group.

A recent randomised control trial of a Schlemm's canal microstent (Hydrus II) with phacoemulsification in patients with open angle glaucoma, assessed 100 patients.[7] Cases were randomised to have either CS alone or CS with microstent implant. In the CS group, washed-out IOP reduced from baseline of 26.6±4.2 to 17.4±3.7 mm Hg at 12 months. The mean IOP reduction was 9.0 mm Hg at 1 year. This study had much higher baseline IOP (26.6±4.2 mm Hg) and consequently, greater observed IOP reduction (9.0 mm Hg) at 1 year, compared with our study (2.0±3.6 mm Hg) and OHTS (4.0 mm Hg).[20] One should take into consideration the effect of regression to the mean phenomenon when interpreting a physiological values such as IOP which is known to fluctuate.[7]

Contrary to our study, Meyer and associates[10] reported no enhancement of the facility of outflow at 6 weeks' post phacoemulsification CS (baseline outflow facility of 0.39±0.23 vs 0.37±0.16 µL/min mm Hg 6 weeks). This may partly be attributable to the inconsistency of pneumatonography technique used in their study, highlighted by their much higher than usual baseline outflow facility value compared with other historical data.[21]

Kee and Moon[22] carried out pneumatonography before and 2 months after phacoemulsification with IOL implantation in 42 patients with cataract. In this study, the IOP reduction was 2.4±0.4 mm Hg at 2 month postoperatively. They demonstrated that outflow facility improved from 0.26±0.01 to 0.30±0.01 µL/min mm Hg 2 months after lens extraction. Although the duration of that study was short, their findings are in agreement with our results at 3 months (0.14±0.07 preoperatively vs 0.18±0.09 µL/min mm Hg at 3 months). It is worth pointing out that the latter study did not report the anterior chamber depth or gonioscopy of their cases prior to the CS. One would assume that considering the higher prevalence of angle closure in Korean population, narrow angle cases might have been included in their study inadvertently, making the effect of the lens removal on IOP reduction and the facility of outflow more exaggerated.

TOF encompasses changes in trabecular outflow facility, as well as any pressure-dependent changes to inflow (pseudofacility) or uveoscleral outflow.[23 24] Nonetheless, in our study, we investigated the change in TOF before and after CS using the same measurement technique, which theoretically means that these potential confounding effects should be less of an issue.

Table 3 This shows TOF enhancement postsurgery at each postoperative visit at 3, 6 and 12 months compared with baseline TOF

	TOF at baseline	TOF at 3 m (µL/min mm Hg), % of enhancement	Comparison made to baseline	TOF at 6 m (µL/min mm Hg), % of enhancement	Comparison made to baseline	TOF at 12 (µL/min mm Hg), % of enhancement	Comparison made to baseline
Cataract group (n=16)	0.15±0.06	0.20±0.09 (15)	P=0.05, 95% CI −0.11 to 0.1	0.22±0.10 (17)	P=0.02*, 95% CI −0.12 to −0.01	0.18±0.07 (10)	P=0.25, 95% CI −0.07 to 0.02
POAG group (n=11)	0.13±0.08	0.15±0.06 (14)	P=0.2, 95% CI −0.07 to 0.01	0.17±0.07 (16)	P=0.05*, 95% CI −0.08 to −0.1	0.17±0.08 (15)	P=0.07, 95% CI −0.09 to 0.006
Overall (n=27)	0.14±0.06	0.18±0.08 (16)	P=0.02*, 95% CI −0.07 to −0.007	0.20±0.09 (15)	P=0.003*, 95% CI −0.09 to −0.02	0.17±0.08 (10)	P=0.04*, 95% CI −0.06 to −0.001

Additionally, the percentage of TOF enhancement is shown.
*Statistically significant.
IOP, intraocular pressure; POAG, primary open angle glaucoma; TOF, tonographic outflow facility.

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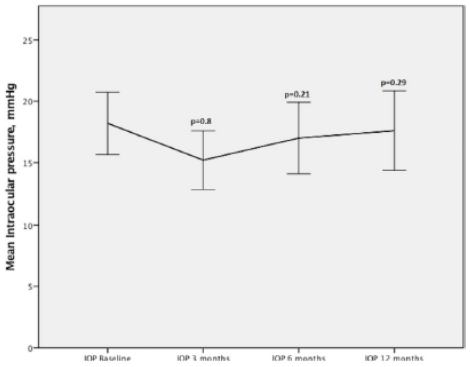

Figure 7 Intraocular pressure changes during postoperative visits in the control group. *Statistically significant.

Per the cellular theory, proposed by Wang *et al*[28] and Tsuboi *et al*,[29] ultrasound can induce chemical and cellular changes in vitro in cultured trabecular meshwork cells. However, another in vivo clinical studies failed to show a linear correlation between ultrasound energy and IOP reduction after CS.[32]

One limitation of our study is that POAG cases should have had a washout period from their glaucoma medications prior to study measurements. However, the number of medications which can affect trabecular outflow facility remained unchanged postoperatively. Furthermore, the results of the subgroup analysis were also similar to that of the combined data. However, because the number of subgroup cases were small, the subgroup analysis should be interpreted with caution.

To our knowledge, this is the first study to assess the TOF after small incision phacoemulsification cataract extraction and IOL implantation using electronic Schiotz tonographer, which is the least variable measurement technique compared with other techniques, with documented anterior chamber depth and gonioscopy. We demonstrated that the IOP drop is accounted for by the increase in TOF. However, the exact mechanism of the outflow facility enhancement remains unclear from this study. Further research is needed to elicit this important question.

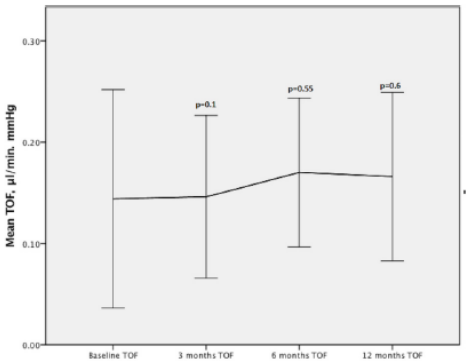

Figure 8 Tonographic outflow facility (TOF) changes during postoperative visits in the control group. *Statistically significant.

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Competing interests None declared.

Patient consent Detail has been removed from this case description/these case descriptions to ensure anonymity. The editors and reviewers have seen the detailed information available and are satisfied that the information backs up the case the authors are making.

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Appendix. Goldmann equation

The aqueous production (flow) rate is equal to the sum of trabecular outflow and uveoscleral outflow

$$Ff = (Pi - Pe).C + Fu$$

where, Ff is aqueous humour flow measured by fluorophotometry, Pi is intraocular pressure in the anterior chamber, Pe is the episcleral venous pressure, C is trabecular outflow facility measured by tonography and Fu is uveoscleral outflow.

In this study, "*C*" was measured at baseline and at 3, 6 and 12 months, while phacoemulsification is assumed to have no effect on *Ff*, *Fu* and *Pe*. We considered *Ff* to be 2.5 µl/min, *Fu* to be 1 µl/min and *Pe* to be 8 mmHg. Therefore, a 10% drop in *Pi*, consistent with the IOP decrease observed at 6 months, should correspond to 18% increase in "*C"*. In addition, a 12% drop in *Pi*, should correspond to 13% increase in "*C*" at 3 months. Furthermore, we observed a 10% drop in *Pi* should correspond to 11% improvement in "*C*" at 12 months.

The PDF copy of the aqueous humour dynamics in uveitic eyes

Aqueous Humor Dynamics in Uveitic Eyes

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• PURPOSE: To investigate aqueous humor dynamics in uveitic eyes.
• DESIGN: Cross-sectional study.
• PARTICIPANTS: Patients with recurrent (≥3 attacks) anterior uveitis (now quiescent) and being treated for glaucoma or ocular hypertension (OHT) (Group 1), previous recurrent anterior uveitis (≥3 attacks) without glaucoma or OHT (Group 2), and normal subjects with no ocular problems and IOP < 21 mm Hg at screening (control group; Group 3).
• METHODS: Patients had one-off measurements. Group 1 patients who were on antihypertensives were washed out for a 4-week period, prior to their study measurements. Main outcome measures were tonographic outflow facility, aqueous humor flow rate, and uveoscleral outflow.
• RESULTS: One hundred and one patients were screened between February 2014 and February 2017. Nine patients did not meet the inclusion criteria. Groups 1 and 3 each included 30 patients, and Group 2 included 32 patients. The mean intraocular pressure was higher in Group 1 compared to the others (25 ± 10.2 mm Hg in Group 1 vs 16 ± 2.7 mm Hg in Group 2 vs 16 ± 2.2 mm Hg in Group 3, $P < .001$). The tonographic outflow facility was lower in Group 1 compared to the others (0.18 ± 0.1 μL/min/mm Hg in Group 1 vs 0.25 ± 0.1 μL/min/mm Hg in Group 2 vs 0.27 ± 0.1 μL/min/mm Hg in Group 3, $P = .005$). However, aqueous humor flow rate was not statistically different (2.47 ± 0.9 μL/min in Group 1 vs 2.13 ± 0.9 μL/min in Group 2 vs 2.25 ± 0.7 μL/min in Group 3, $P = .3$). There was also no significant difference in calculated uveoscleral outflow.
• CONCLUSION: This is the first aqueous humor dynamics study in patients with uveitic glaucoma/OHT and recurrent anterior uveitis compared with age-matched controls. We have demonstrated that the elevated intraocular pressure seen in the uveitic glaucoma/OHT eyes (3-6 attacks) was due to reduced tonographic outflow facility. The aqueous humor flow rate was not detectibly different, nor did the calculated uveoscleral outflow demonstrate any discernible difference. However, the exact mechanism remains to be elucidated. (Am J Ophthalmol 2019;■:■–■. © 2019 Elsevier Inc. All rights reserved.)

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UVEITIS IS ONE OF THE MOST COMMON ophthalmic conditions seen in eye departments globally. The prevalence of uveitis varies in different parts of the world[1–3]; however, idiopathic recurrent anterior uveitis[4,5] is the most common diagnosis in those affected. One of the most serious sight-threatening sequelae from this condition is uveitic glaucoma, which has a reported incidence of between 5% and 24%[6–8] among long-term uveitic eyes. Despite the relatively high prevalence, there have only been a few previous aqueous humor dynamics studies exploring the pathogenesis of uveitic glaucoma in humans. This is partly owing to measurement challenges, which are unfortunately quite common in uveitic glaucoma/ocular hypertension (OHT) cases. Firstly, eyes with prior intraocular surgery, such as cataract surgery, glaucoma filtration surgery, or iridotomy/iridectomy, must be excluded. This is owing to iridolenticular barrier compromise, which subsequently may lead to excessive posterior flow of fluorescein and thereby disrupt the fluorescein loss assumptions underlying the fluorophotometry measurements.[9,10] Secondly, active uveitis can render the fluorophotometry measurements inaccurate. This is because of the presence of excessive protein in the anterior chamber, which can bind with the fluorescein molecule and disrupt its natural clearance from the anterior chamber.[9,10]

Considering these limitations, it is not surprising that there have only been 2 previous human aqueous humor dynamics studies in uveitis or uveitic glaucoma/OHT and both were in those with active uveitis. The first study was by Ladas and associates,[11] who only investigated the correlation between tonographic outflow facility measured by Schiøtz tonography and laser flare photometry. However, they did not measure the aqueous humor flow rate. Their cohort comprised patients with active uveitis (any type of uveitis including panuveitis and posterior uveitis were included). None of the eyes had any history of raised intraocular pressure (IOP). They showed that higher flare measurement in the anterior chamber coincided with lower outflow facility.

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The other study, by Johnson and associates,[12] compared tonographic outflow facility (Schiøtz) and aqueous humor flow rate (fluorophotometry) in 10 eyes with cyclitis to their fellow healthy eyes. The results showed that the aqueous humor flow rate and facility of outflow were not significantly different compared to unaffected fellow eyes. Only 3 cases had raised IOP at the time of the measurement. They also had some anterior chamber activity (trace to 2+ cells and flare in the anterior chamber was reported), which may have compromised the accuracy of the tonographic outflow facility and aqueous humor flow rate measurements.

With regard to aqueous humor dynamics, there are probably 3 distinct circumstances where uveitis can lead to raised IOP: (1) those eyes that are currently actively inflamed, with anterior chamber inflammatory cells and proteins as well as concurrent steroid use; (2) previous severe or multiple uveitis attacks with a high likelihood of having had incisional ocular surgeries such as glaucoma filtration or cataract surgeries (such as juvenile chronic arthritis associated uveitis); (3) previous multiple but moderate anterior uveitis attacks that are currently quiescent.

As fluorophotometry can be inaccurate in eyes that are actively inflamed, we have therefore designed this study specifically investigating quiescent eyes of the last category of the aforementioned list. This helps us understand why some eyes develop uveitic glaucoma/OHT after only a few attacks of anterior uveitis and not others.

METHODS

THIS STUDY IS A CROSS-SECTIONAL STUDY COMPARING aqueous humor dynamics in age-matched healthy volunteers, subjects with previous anterior uveitis (without glaucoma), and those with uveitic glaucoma/OHT but no active uveitis at the time of study measurement.

Ethical approval for this study was obtained from the local St Thomas' Hospital research ethics committee. This research followed the tenets of the Declaration of Helsinki (http://www.clinical-trials.gov, identifier NCT02765308, May 6, 2016). Healthy volunteers were recruited from the hospital staff and their family members after having a comprehensive ophthalmic examination. Patients with recurrent anterior uveitis were identified from our uveitis and glaucoma clinics at St Thomas' Hospital, London, United Kingdom. A patient information leaflet was provided at the initial contact, and signed informed consent was obtained before glaucoma eye drop washout and study measurements took place. Participants enrolled in the study were divided into 3 groups: (1) previous recurrent anterior uveitis (≥3 attacks) with glaucoma/OHT (uveitic glaucoma) on topical glaucoma drops treatment (Group 1); (2) previous recurrent anterior uveitis (≥3 attacks) with normal IOP and no diagnosis of glaucoma (Group 2); and (3) healthy volunteers (Group 3). All uveitic eyes were quiescent at the time of enrollment (defined by absence of any flare/cells on slit-lamp biomicroscopy). All patients in the uveitic glaucoma/OHT group had a 4-week washout period from their antihypertensive and steroid treatments prior to their study measurements (patients had a safety visit 2 weeks after commencing the washout).

• **ELIGIBILITY CRITERIA:** Inclusion criteria were as follows: age >18 years, adequate cognitive function and ability to understand verbal and written information in English, and previous recurrent anterior uveitis (≥3 attacks) with/without OHT/glaucoma. Exclusion criteria were other glaucoma diagnosis, including pigment dispersion syndrome and pseudoexfoliation; active uveitis; ocular trauma; intraocular or keratorefractive surgery; use of systemic medication that may affect aqueous humor production, such as beta-blockers; a history of allergy or hypersensitivity to fluorescein; and any abnormalities preventing reliable IOP or fluorophotometric readings.

• **PRIMARY OUTCOME MEASURES:** Primary outcome measures were facility of tonographic outflow (measured by digital Schiøtz tonometry), aqueous humor flow rate (measured by fluorophotometry), and uveoscleral outflow (calculated from the Goldmann equation).

• **MEASUREMENTS:** All patients underwent clinical ophthalmic examinations including visual acuity, slit-lamp biomicroscopy, gonioscopy, anterior chamber depth, axial length measurement (IOLMaster; Carl Zeiss Meditec Inc, Dublin, California, USA), central corneal thickness (Pachmate DGH 55; DGH Technology, Inc, Exton, Pennsylvania, USA), visual fields (Humphrey automated white-on-white, 24-2 SITA-standard; Carl Zeiss Meditec), and dilated ophthalmoscopy. 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 (aged <26 years, 5-6 drops; 26-35 years, 4 drops; >35 years, 3 drops).[13] Fluorophotometry was performed in both eyes with a scanning ocular fluorophotometer (FM-2, Fluorotron Master ocular fluorophotometer; OcuMetrics, Mountain View, California, USA) from 9 AM to 12 noon. The aqueous humor flow rate was determined using dedicated software provided with the fluorophotometer. Duplicate or triplicate scans were collected and repeated at 1-hour intervals for 4 measurements to determine the aqueous humor flow rate. Following each set of scans, IOP was measured using pneumotonometry (Model 30 Classic; Reichert Ophthalmic Instruments, Depew, New York, USA); IOP was recorded as the arithmetic mean of a total of 12 measurements per eye (3 measurements every hour alternating between eyes).

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Tonographic outflow facility (C) was measured by constant-weight tonography (5.5, 7.5, or 10 g) using a modified digital Schiøtz tonographer (designed by the Department of Bioengineering, Imperial College, London, UK) at 10-11 AM. Our device used an original Schiøtz tonographer footplate from a commercially available unit (model 720; Berkeley Bioengineering Inc, San Leandro, California, USA) attached to a 3D printed shell that was designed such that the weight conformed to the specifications set out by the Committee on Standardization of Tonometers.[14] Displacement of the weighted plunger was measured using a linear variable differential transformer (MHR, TE Connectivity, Schaffhausen, Switzerland) driven by a signal conditioner (AD698; Analog Devices, Norwood, Massachusetts, USA) and captured digitally by a data acquisition system (USB-6009; National Instruments, Austin, Texas, USA). Validation studies confirmed that the linear variable differential transformer voltage output was linear with respect to the Schiøtz scale reading (Supplemental Figure 1; Supplemental Material available at AJO.com), where each scale reading is equivalent to 0.05 mm of plunger displacement.[14] Facility was estimated using Grant's equation (Equation 1)[15]:

$$C = \frac{V_{c,t} - V_{c,0} + \frac{1}{K}(\log P_{t,0} - \log P_{t,t})}{\left(\frac{P_{t,0}+P_{t,t}}{2} - P_0 - \Delta P_v\right) t} \qquad \text{Equation 1}$$

where $V_{c,t}$ and $V_{c,0}$ are the aqueous volumes displaced at time t and at the start of tonography ($t = 0$); $P_{t,t}$ and $P_{t,0}$ are values of IOP at time t and at the start of tonography; P_0 is the IOP immediately prior to the start of tonography; ΔP_v is the change in episcleral venous pressure, assumed to be 1.25 mm Hg[16]; and K is the coefficient of ocular rigidity, assumed to be 0.0215/μL^{-1}.[17] $V_{c,t}$, $V_{c,0}$, $P_{t,t}$, $P_{t,0}$, and P_0 were determined based on the value of the Schiøtz scale reading and tables provided by Moses and Becker.[18] By minimizing the root mean square error between the tonographic tracing and Equation 1 (Supplemental Figure 2; Supplemental Material available at AJO.com), the optimal value of C was determined numerically.

At present the clinical measurement of uveoscleral outflow in humans is not possible[19]; hence this value is generally calculated from the Goldmann equation. Sit and McLaren used a computerized venomanometry to measure episcleral venous pressure (EVP).[20] They illustrated that EVP in normal subjects can vary between 6 and 10 mm Hg. Therefore, we have used this EVP range for our calculations. However, to make this calculation valid, it must be assumed that the episcleral venous pressure did not vary significantly between all 3 groups of patients in our study.

Uveoscleral outflow was calculated using the Goldmann equation (Equation 2) with an assumed episcleral venous pressure of 6-10 mm Hg.

Ff is the rate of aqueous humor formation measured by fluorophotometry, C is the tonographic facility of outflow, Pi is the intraocular pressure, Pe is the episcleral venous pressure, and Fu is uveoscleral flow.

$$Ff = (Pi - Pe)C + Fu \qquad \text{Equation 2}$$

Therefore,

$$Fu = Ff - C(Pi - Pe) \qquad \text{Equation 3}$$

Only 1 randomly (Excel random number generator; Microsoft, Redmond, Washington, USA) chosen eye per participant was included in the data analysis, when both eyes fulfilled the inclusion criteria.

• **SAMPLE SIZE CALCULATION:** The sample size estimate was based on the results of paired measurements of 2 parameters (aqueous flow and facility of outflow) in a previous study done at the Mayo Clinic, Rochester, Minnesota, USA, by the chief investigator (K.S.L.).[21] This study had a 90% chance of finding a 5% difference in IOP, 5.4% difference in aqueous flow, and 7.5% difference in outflow facility among medication groups, if these differences existed ($n = 30$ subjects, $\alpha = 0.05$, and $\beta = 0.10$).

• **DATA ANALYSIS:** Histograms and 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 t test was 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. Linear regression analyses were used to determine the correlation of one parameter vs another parameter of aqueous humor dynamics. $P < .05$ was considered statistically significant (all analyses, SPSS 24.0; SPSS, Chicago, Illinois, USA).

RESULTS

WE SCREENED 101 PATIENTS BETWEEN FEBRUARY 2014 AND February 2017. Nine patients did not meet the inclusion criteria. Thirty patients with recurrent anterior uveitis and being treated for secondary glaucoma/OHT (Group 1) and 32 patients with previous recurrent anterior uveitis (Group 2) without OHT or glaucoma and with normal IOP who met the inclusion/exclusion criteria were recruited. Thirty healthy volunteers were enrolled as controls (Group 3) over the same period. There was a female preponderance in Groups 2 and 3. Most subjects were white; there were a few black African/Caribbean and Asian subjects in Groups 2 and 3 but slightly higher presence of black patients in Group 1 ($P = .06$). The mean age in all 3 groups was comparable ($P = .3$). The best-corrected visual acuity in the

TABLE 1. Baseline Characteristics of All Participants in Study of Aqueous Humor Dynamics in Uveitic Eyes

	Group 1 [Uveitic Glaucoma/OHT] (n = 30)	Comparison Between Group 1 and Group 3		Group 2 [Uveitis] (n = 32)	Comparison Between Group 1 and Group 2		Group 3 [Normal] (n = 30)	Comparison Between Group 2 and Group 3	
		P Value	95% CI		*P* Value	95% CI		*P* Value	95% CI
Sex (F: M)	14 (47%): 16 (53%)	.06	—	24 (80%): 8 (20%)	.06	—	20 (67%): 10 (33%)	.06	—
Ethnicity (W: B: A)	10: 13: 7	.01	—	22: 9: 1	.01	—	21: 5: 4	.01	—
Age, years	50 ± 14.0	.9	−7.2 to 8.4	49 ± 11.1	.4	−12.3 to 3.4	44 ± 13.6	.3	−12.8 to 2.7
BCVA, logMAR	0.04 ± 0.2	.02*	−0.2 to −0.01	−0.06 ± 0.1	.002*	−0.2 to −0.04	−0.09 ± 0.1	.7	−0.1 to 0.05
CCT, µm	552 ± 37.0	.6	−27.5 to 11.6	544 ± 31.2	.7	−26.0 to 13.8	546 ± 28.2	.9	−17.7 to21.4
ACD, mm	3.34 ± 0.3	.6	−0.3 to 0.12	3.26 ± 0.4	.3	−0.1 to 0.4	3.49 ± 0.3	.06	−0.02 to 0.4
AXL, mm	23.4 ± 4.0	.8	−1.2 to 1.9	23.8 ± 1.4	.5	−0.8 to 2.3	24.2 ± 1.5	.8	−1.2 to 1.9
MD, dB	−3.79 ± 6.1	.1	−0.3 to 4.3	−1.80 ± 2.4	.05*	0.03 to 4.8	−1.36 ± 1.8	.9	−1.9 to 2.7

Asterisk (*) indicates statistically significant values.
A = Asian; ACD = anterior chamber depth; AXL = axial length; B = black; BCVA = best-corrected visual acuity; CCT = central corneal thickness; CI = confidence interval; MD = mean deviation; OHT = ocular hypertension; W = white.

uveitic glaucoma/OHT (Group 1) was worse compared to other groups ($P = .002$). This was primarily owing to lens opacity and severity of glaucoma in Group 1. Anterior chamber depth, axial length, and central corneal thickness were similar across all groups ($P = .3$). Visual field parameters were worse in Group 1 compared to the other 2 groups ($P = .05$). All patients had open angles on gonioscopy; only 5 (17%) patients in Group 1 had noncontiguous patchy peripheral anterior synechiae. The subjects' baseline characteristics are summarized in Table 1.

Mean IOP was significantly higher in the uveitic glaucoma/OHT group compared to the other 2 groups ($P < .001$). The tonographic outflow facility (C) was markedly lower in the uveitic glaucoma/OHT group compared to the other 2 groups ($P = .005$).

However, aqueous humor flow rate was not statistically different between the 3 groups: 2.46 ± 0.9 µL/min (Group 1) vs 2.13 ± 0.9 µL/min (Group 2) vs 2.25 ± 0.7 µL/min (Group 3) ($P = .3$). Additionally, no significant difference in uveoscleral outflow (with assumed episcleral venous pressure of 6-10 mm Hg based on Sit and McLaren's work[20]) was detected between the 3 groups, even when allowing episcleral venous pressure to vary over the range of 6-10 mm Hg (but assuming a uniform pressure for all patients) ($P = .9$). The full aqueous humor dynamic results are shown in Table 2.

A breakdown of different uveitis diagnoses in Groups 1 and 2 are provided in Table 3. Most cases were idiopathic anterior uveitis.

The study eyes in Group 1 on average had 4.5 episodes (range 3-6) of uveitis attacks while eyes in Group 2 had on average 6 episodes (range 3-10) prior to study measurements.

If, rather than including all individuals in the uveitic glaucoma/OHT group as in Table 2, we excluded 14 individuals who maintained a normal IOP (<21 mm Hg) post washout, the average IOP in the uveitic glaucoma/OHT group increased to 31.8 ± 9.7 mm Hg, which was greater than either the uveitis group (Group 2; 16 ± 2.5 mm Hg) or control group (Group 3; 16 ± 2.2 mm Hg) ($P < .001$). Correspondingly, the tonographic outflow facility in the uveitic glaucoma/OHT group, excluding those with normal IOP post-washout, was 0.13 ± 0.1 µL/min/mm Hg, which was significantly lower than either the uveitis group (Group 2; 0.27 ± 0.1 µL/min/mm Hg) or control group (Group 3; 0.25 ± 0.1 µL/min/mm Hg) ($P < .001$). Nonetheless, even after removing those with normal postwashout IOP, there was still no statistically significant difference in aqueous humor flow rate between 3 groups (2.42 ± 0.9 µL/min, Group 1 vs 2.18 ± 0.9 µL/min, Group 2 vs 2.32 ± 0.8 µL/min, Group 3, $p = .4$). Similarly, the uveoscleral outflow was comparable between all groups: -0.01 ± 2.06 vs 0.64 ± 1.3 in uveitis (Group 2) vs 0.75 ± 1.4 in controls (Group 3), $P = .1$.

Correlation was made between IOP and either aqueous humor flow rate, tonographic outflow facility, uveoscleral

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TABLE 2. Aqueous Humor Parameters and Comparison Made Between All 3 Groups

	Group 1 [Uveitic Glaucoma/OHT] (n = 30)	Comparison Between Group 1 and Group 3		Group 2 [Uveitis] (n = 32)	Comparison Between Group 1 and Group 2		Group 3 [Normal] (n = 30)	Comparison Between Group 2 and Group 3	
		P Value	95% CI		*P* Value	95% CI		*P* Value	95% CI
IOP, mm Hg (min-max)	25 ± 10.2 (10-52)	<.001*	−12.5 to −4.6	16 ± 2.5 (10-22)	<.01*	4.3-12.5	16 ± 2.2 (11-19)	.9	−3.6 to 4.7
Aqueous flow rate(Ft), µL/min	2.47 ± 0.9	.3	−0.8 to 0.21	2.18 ± 0.9	.7	−0.66 to 0.37	2.32 ± 0.8	.7	−0.36 to 0.65
Trabecular outflow facility(C), µL/min/mm Hg	0.18 ± 0.1	.005*	0.02-0.15	0.27 ± 0.1	.04*	0.01-0.14	0.25 ± 0.1	.7	−0.08 to 0.04
Uveoscleral outflow (Fu) 10, mm Hg[a]	0.49±1.6	0.9	−0.73 to 1.02	0.64 ± 1.3	.7	−0.62 to 1.15	0.75 ± 1.4	.9	−0.75 to 0.99

Asterisk (*) indicates statistically significant values.
IOP = intraocular pressure; OHT = ocular hypertension.
[a]Calculated uveoscleral outflow assumed episcleral venous pressure of 10 mm Hg.

outflow, anterior chamber depth, or axial length. Only the correlation between postwashout IOP and tonographic outflow facility was statistically significant, with a strong negative correlation observed in Group 1 ($R^2 = 0.86$, $P < .001$). The other correlations were not significant ($P > .09$) (Figure). However, correlation between IOP and aqueous humor parameters was not significant in other groups ($P = .6$).

DISCUSSION

THIS IS THE FIRST AQUEOUS HUMOR DYNAMICS STUDY IN patients with uveitic glaucoma/OHT and recurrent anterior uveitis compared with age-matched healthy controls. However, one should be mindful of the fact that uveoscleral outflow was calculated by the Goldmann formula using assumed episcleral venous pressure. Additionally, we used indirect techniques to measure the aqueous humor flow rate and tonographic outflow facility. Consequently, these parameters may have been compromised by subclinical inflammation. The uveitis and uveitic glaucoma/OHT cases were all clinically quiescent at the time of enrollment and none in the uveitic glaucoma/OHT group had more than 6 previous anterior uveitis attacks. We demonstrated that the elevated intraocular pressure seen in the uveitic glaucoma/OHT eyes was owing to reduced tonographic outflow facility (this is used as a proxy of measuring trabecular outflow facility[22]). The aqueous flow rate was not detectibly different among the 3 groups, nor did the calculated uveoscleral outflow demonstrate any difference between 3 groups.

This study is unique in several aspects. Firstly, it encompasses age-matched healthy controls as well as those with previous recurrent anterior uveitis with or without glaucoma/OHT. Additionally, in this study, previously treated uveitic glaucoma/OHT patients underwent a 4-week washout period from their glaucoma medications, mydriatics, and steroids before the study measurements. It is therefore suggestive that the reason for raised IOP, after a moderate number of recurrent anterior uveitis attacks, is caused by increased tonographic outflow resistance without significant impairment to aqueous humor production.

Although the reduction in tonographic outflow facility in our finding should not come as a surprise (as in almost all other types of glaucoma, tonographic outflow impairment is the primary cause of raised IOP[23]), this is the first study to confirm this in uveitic glaucoma. Ladas and associates[11] investigated the correlation between outflow facility measured by Schiøtz tonography and laser flare photometry in patients with active uveitis. They demonstrated that the higher the measured flare in the anterior chamber (>20 photon units/ms), the lower the outflow facility (0.21 ± 0.12 µL/min/mm Hg). They also reported

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TABLE 3. Uveitis Diagnosis in Groups 1 and 2

	Idiopathic	Sarcoidosis	HLA-B27 Associated	Psoriasis	Tuberculosis Related	Herpetic
Group 1 (n = 30)	21	4	3	0	1	1
Group 2 (n = 32)	22	2	7	1	0	0

that patients with flare <20 photon units/ms had a similar outflow facility to normal controls. Although increased aqueous protein level may lead to obstruction of trabecular meshwork pores in the acute phase, it may also have lasting effect on outflow facility, as demonstrated by Epstein and associates.[24] They explored the facility of outflow in enucleated human eyes by infusing the eyes with human plasma and showed that facility of outflow reduced by over 40% and that, interestingly, this was not resolved by irrigating the eyes with balanced salt solution. The authors speculated that this may be owing to adhesion of serum components of plasma to the aqueous outflow system. All uveitic glaucoma/OHT eyes in our cohort had quiescent anterior chamber on slit-lamp examination (although they may have had subclinical inflammation) at the time of measurements; however, there might have been lasting damage to trabecular meshwork owing to repeated anterior chamber inflammation or even previous long-term use of topical steroids, which can eventually cause compromised outflow and, consequently, raised IOP. Mechanical obstruction owing to peripheral anterior synechiae could also account for some of the reduced outflow facility. The evidence from animal studies suggests that inflammatory cells can cause blockage of outflow facility by simply clogging the trabecular meshwork pores.[25] Chronic inflammation of the trabecular meshwork may lead to scar formation and permanent damage to the underlying tissue.[26] In a recent multicenter study of risk factors of ocular hypertension in noninfectious uveitis,[6] the presence of peripheral anterior synechiae (PAS) carried a 3-fold risk of developing OHT, whereas in our study only 17% of uveitic glaucoma/OHT cases had some degree of noncontiguous PAS and none had more than 180 degrees of PAS, suggesting that raised IOP might have been due to microstructural damage to the trabecular meshwork. Additionally, extracellular matrix accumulation in the trabecular meshwork or increased continuity of the endothelial basement membrane along the Schlemm canal, coinciding with long-term use of steroids, may play a part in obstruction of trabecular outflow and, subsequently, raised IOP.[27,28]

Calculated uveoscleral outflow in our present study in human uveitic glaucoma/OHT (as a noninvasive direct clinical measurement remains elusive) is in marked contrast to uveoscleral outflow measured in monkeys' eyes with active uveitis by Toris and Pederson.[29] In their study using cynomolgus monkeys, the anterior uveitis was artificially induced with intracameral injection of albumin. The aqueous flow rate and uveoscleral outflow was measured using fluorescein isothiocyanate dextran 70. They found that the uveoscleral outflow was 4 times greater in the inflamed eyes than the controlled eyes. It is therefore likely that in actively inflamed eyes, with the edematous ciliary body and suprachoroidal space seen in the monkeys' eyes, as well as the release of endogenous prostaglandins,[30] there will be a transient increase in uveoscleral outflow, which subsided once the inflammation had settled, as in our study. Based on this observation, the authors would like to speculate that topical prostaglandin is more likely to be effective in lowering IOP in the quiescent eye than in the active uveitis eye.

Postoperative hypotony after glaucoma filtration surgery in uveitic glaucoma has been routinely attributed to "aqueous shutdown" without any evidence to substantiate this claim.[31–33] Therefore, one of the most interesting findings in our study is the similar level of aqueous production rate in all 3 groups. This suggests that after fewer than 7 attacks of anterior uveitis, there may not be any significant damage to the ciliary epithelium and other associated apparatus involved in the production of aqueous humor in human eyes. We limited our uveitis groups to only those eyes with more than 3 attacks of anterior uveitis and, interestingly, none of our recruited cases in Group 1 had more than 6 attacks of uveitis. We identified many other cases of uveitic glaucoma with more than 6 previous attacks, but none were eligible for our study owing to previous intraocular surgeries, such as cataract and glaucoma surgeries. It is therefore plausible that uveitic glaucoma patients who have more than 6 attacks of uveitis may have different aqueous dynamic parameters, including aqueous production rate change.

Based on available evidence and our own study, aqueous production rate is probably only reduced in those cases of severe acute uveitis or those eyes with previous multiple (more than 6) and severe uveitis attacks, such as those associated with idiopathic juvenile arthritis. Therefore, surgical techniques,[34] rather than "aqueous shutdown," are the most likely cause of hypotony post glaucoma filtration surgeries, in those glaucoma eyes with moderate uveitis.

As part of the washout process before the aqueous humor dynamic measurements, we also observed an interesting finding in our study that may have significant clinical ramifications. After washout, the mean IOP in nearly half (47%) of the uveitic glaucoma/OHT group was less than 22 mm Hg. It is likely that following a period of inactivity of the uveitis as well

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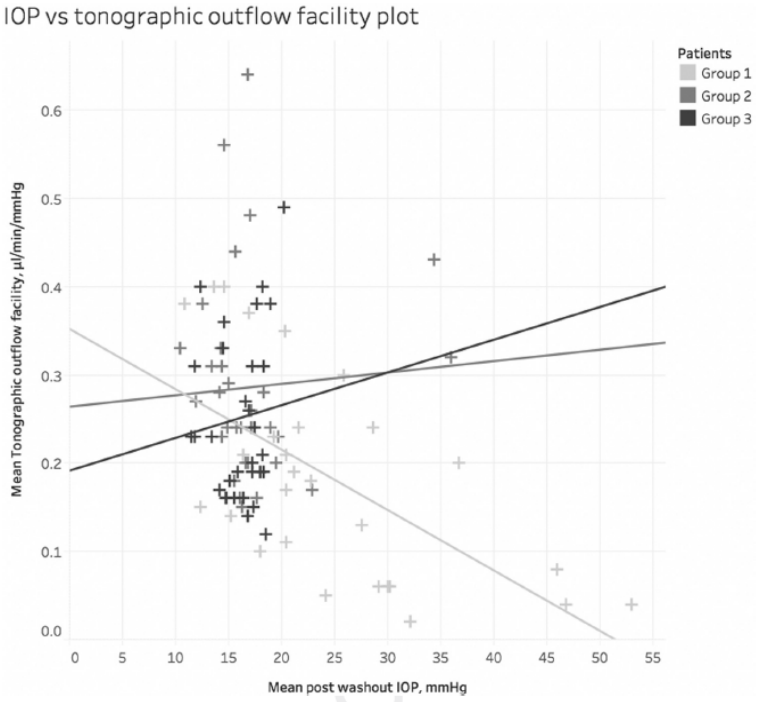

FIGURE. Intraocular pressure (IOP) vs tonographic outflow facility plot. Correlation was made between postwashout IOP and tonographic outflow facility.

as cessation of topical steroid treatment, in those cases of presumed uveitic glaucoma/OHT, IOP may revert to normal after washout. Clinically, it is therefore sensible to consider treatment washout in this group of patients after these eyes have been controlled on glaucoma drops treatment after 6-12 months without any recurrence of their uveitis.

There are, however, a few inherent limitations in aqueous humor dynamics studies. The most important of all is that the eyes undergoing aqueous humor dynamic measurements should not have had any intraocular surgery such as cataract surgery or iridotomy, which may compromise the iridolenticular barrier to the posterior flow of fluorescein during fluorophotometry measurement.[9,10] Active uveitis also renders the fluorophotometry measurement inaccurate owing to the presence of excessive protein in the anterior chamber, which can bind to fluorescein molecules. Furthermore, with the breakdown of the blood-aqueous barrier, fluorescein can diffuse through unconventional pathways, potentially distorting the assumptions about the standard diffusional loss of fluorescein during fluorophotometry.[9,10] Tonographic outflow facility is influenced by "pseudofacility," especially in uveitis, owing to possible subclinical inflammation.[22] Therefore, there might be a discordance between tonographic outflow facility and trabecular outflow facility. Another issue relates to the measurement of episcleral venous pressure and uveoscleral outflow. At present the precise measurement of uveoscleral outflow is not possible in humans[19]; hence this value is generally calculated from the Goldmann equation. As we are also unable to accurately measure the EVP[21,35] despite some experimental methods of measuring EVP,[20] they are not widely available. Therefore, it is generally accepted that EVP is approximately 10 mm Hg in humans. To make this calculation valid, it must be assumed that the EVP in these 3 groups of patients did not vary significantly. We did not perform flare measurement in our patients as we did not have the flare meter in our department; however, as we have taken great care in excluding any eyes with active uveitis, we do not believe that this measurement will affect the main findings of our study.

In summary, to our knowledge this is the first aqueous humor dynamics study in patients with previous recurrent anterior uveitis and uveitic glaucoma/

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OHT compared with age-matched healthy controls. We have demonstrated that elevated intraocular pressure seen in the uveitic glaucoma/OHT eyes (after less than 7 previous attacks of uveitis) was owing to reduced tonographic outflow facility alone. The aqueous humor flow rate was not detectibly different among the 3 groups, nor did the calculated uveoscleral outflow demonstrate any detectible difference between the 3 groups. Clinicians should also consider treatment washout for those with medically treated uveitic glaucoma in the future. However, future studies should be undertaken once we have better techniques of aqueous dynamics measurements in eyes with active uveitis and previous intraocular surgeries, as well as noninvasive and accurate techniques for measuring EVP and uveoscleral outflow.

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The Effect of High-Intensity Focused Ultrasound on Aqueous Humor Dynamics in Patients with Glaucoma

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Purpose: To investigate the effects of high-intensity focused ultrasound (HiFU) on aqueous humor dynamics in patients with glaucoma.

Design: Comparative, nonrandomized, interventional study.

Participants: Adult patients with a diagnosis of open-angle glaucoma or ocular hypertension with suboptimal intraocular pressure (IOP) control despite maximum medical treatment who required further IOP optimization.

Methods: All patients underwent comprehensive ophthalmic examination before aqueous humor dynamics study measurements, including fluorophotometry and digital Schiøtz tonography. All patients received 6 seconds of HiFU therapy. Aqueous humor dynamics studies were repeated 3 months after the treatment (patients had 4-week washout from their glaucoma medication before their aqueous humor dynamics study measurements at baseline and the 3-month visit).

Main Outcome Measures: Intraocular pressure, facility of topographic outflow, aqueous flow rate, and uveoscleral outflow.

Results: Thirty eyes of 30 patients were included in the study. At the 3-month postoperative visit, the mean postwashout IOP was reduced by 16% (31.7±5.3 vs. 26.6±4.8 mmHg, $P = 0.004$), and aqueous flow rate was decreased by 15% (2.07±0.73 vs. 1.77±0.55 μl/min, $P = 0.05$) from baseline. Neither the tonographic outflow facility nor the uveoscleral outflow was significantly different from baseline. There is a 20% risk of treatment failure (those who needed further glaucoma surgical intervention) within 1 month after a single HiFU treatment ($n = 6$). Only 25 patients (80%) were able to undergo post-treatment washout measurements, and in these eyes, only 26.6% of eyes achieved >20% IOP reduction at 3 months compared with baseline.

Conclusions: We investigated the aqueous humor dynamics effects of a cyclodestructive procedure and specifically HiFU in patients with uncontrolled open-angle glaucoma on maximum tolerated medical therapy. High-intensity focused ultrasound reduced IOP 3 months postoperatively by 16% and aqueous flow decreased by 15% without any significant effect on tonographic outflow facility and uveoscleral outflow. *Ophthalmology Glaucoma 2020;3:122-129 © 2019 by the American Academy of Ophthalmology*

Supplemental material available at www.ophthalmologyglaucoma.org.

Cyclodestructive procedures for glaucoma were introduced into clinical practice approximately a century ago.[1] Their primary aim is to ablate the ciliary epithelium, which produces aqueous humor to reduce intraocular pressure (IOP). Various modalities have been used to achieve this goal, including diathermy,[1] surgical excision,[2] cryotherapy,[3] and laser.[4] Laser is now the most popular treatment method, using an 810-nm diode laser, delivered as trans-scleral diode photocoagulation[5,6] or endoscopically via endocyclophotocoagulation.[7,8]

The other treatment option is to use ultrasound energy to modulate ciliary epithelium function. High-intensity focused ultrasound (HiFU) was first used to treat brain pathologies, such as Parkinson's disease, in the 1940s.[9] The theoretical advantage of this technology is to have the treatment area focused on a well-defined section at a preset depth, thus limiting damage to surrounding tissues.[9] This technology was later used in ophthalmology practice to treat glaucoma in the 1980s. Lizzi et al[10] and Coleman et al[11] conducted studies using a commercially available device: the Therapeutic Ultrasound System (Sonocare Inc, Ridgewood, NJ). They evaluated the efficacy and safety of HiFU in patients with uncontrolled IOP and advanced glaucoma[10,12] with reasonable results. A similar outcome was later confirmed by another clinical study by Sterk et al.[13] However, despite the encouraging initial evidence, the significant risk of complications, such as scleral staphyloma and perforation, corneal thinning, persistent hypotony, phthisis bulbi, and loss of visual acuity, led to abandonment of the procedure in the middle of the 1990s.[13-15] Additionally, the bulky design and complexity of the procedure were among other reasons for not pursuing

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this procedure further.[16] By refining the transducer design and the modes of energy delivery, Eye Tech Care (Rillieux-la-Pape, France) introduced a new ultrasound cycloplasty device using HiFU technology in 2011, called the "EyeOP1."[17] This device received the CE mark in 2011 and Chinese Food and Drug Administration approval in 2017. The device delivers ultrasound energy to the ciliary body with preset parameters. This device consists of a console, treatment probe, and coupling cone. The probe consists of 6 miniature piezoelectric curved areas inside the probe, which create well-circumscribed areas of treatment on the eye. The high frequency enables focused delivery of ultrasound. The focusing area is not more than 0.1 mm by 1 mm. The device preset parameters are 21 MHz frequency and 2.45 W acoustic power with an activation period of 8 seconds. The HiFU probe is supplied in 3 sizes (11, 12, and 13 mm), which fit most ocular sizes, and for every patient, the choice of the right size is based directly on white-to-white and axial length measurements.[18]

Initial reports suggested that HiFU has a comparable mechanism of action as other cyclodestructive procedures such as trans-scleral diode photocoagulation, which lowers IOP by destroying ciliary processes and suppressing aqueous production.[18,19] However, Coleman et al[20] speculated that the focused ultrasound may have dual effects in reducing IOP by increasing outflow through the sclera from the induced scleral thinning. Mastropasqua et al[21] used anterior segment OCT and in vivo confocal microscopy. They indirectly assessed the uveoscleral outflow pathway via scleral changes (as a surrogate for uveoscleral outflow pathway) post-HiFU treatment in patients with glaucoma. They found increased intrascleral hyporeflective spaces in anterior-segment OCT. These authors contemplated that these findings likely led to an increase in uveoscleral outflow.

The aqueous humor dynamic effects of HiFU treatment have never been studied. We have designed this case-control study to assess the effects of HiFU on aqueous humor parameters and confirm the exact mechanism of action.

Methods

Ethics approval for this study was obtained from the National Health Service research ethics committee, United Kingdom. This research conformed to tenets of the Declaration of Helsinki (https://Clinicaltrials.gov identifier NCT02839590; National Institute for Health Research portfolio registration number 35357). Consecutive patients with glaucoma or ocular hypertension (OHT) (glaucoma was defined as open-angle glaucoma cases including pigmentary glaucoma diagnosed on the basis of abnormal visual field testing and corresponding disc changes diagnosed by a glaucoma specialist) and suboptimal IOP control despite maximum medical treatment were invited to participate in the study. A patient information leaflet was provided at the initial contact, and signed consent was obtained before the measurements and treatment were carried out.

Eligibility Criteria

All of the following inclusion criteria were met:

- All patients aged 18 to 90 years
- Diagnosis of open-angle glaucoma or OHT with suboptimal IOP control despite maximum medical treatment
- Ability to undergo accurate fluorophotometry and tonography

Patients were excluded if they had any of the following:

- Mental impairment conflicting with informed consent or follow-up
- Allergy to fluorescein
- Current use of any investigational drug or device, or current participation in an interventional clinical trial
- Previous intraocular incisional surgeries, including iridotomies, cataract surgeries, or glaucoma filtration surgery

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

Patients then underwent a 4-week washout period from all glaucoma treatments before baseline study measurements (patients had a safety visit 2 weeks postwashout). The washout process was repeated at the 3-month visit before study measurements (with a safety 2-week postwashout visit).

The night before the baseline study visit (10 PM) for the fluorophotometric scans, participants self-administered 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 $\leq$25 years, 5–6 drops; age 26–35 years, 4 drops; $\geq$35 years, 3 drops).[22] 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. 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 pneumatonometry (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 2 eyes.

Tonographic outflow facility (C) was measured by constant weight tonography (5.5–10 g) using a modified digital Schiøtz tonographer (designed by the Department of Bioengineering, Imperial College London, London, UK) at 10 to 11 AM. Our device used an original Schiøtz tonographer footplate from a commercially available unit (model 720, Berkeley Bioengineering Inc, San Leandro, CA) attached to a 3-dimensional printed shell that was designed such that the weight conformed to the standards set out by the Committee on Standardization of Tonometers.[22] Displacement of the weighted plunger was measured using a linear variable differential transformer (MHR, TE Connectivity, Schaffhausen, Switzerland) driven by a signal conditioner (AD698, Analog Devices, Norwood, MA) and captured digitally by a data acquisition system (USB-6009, National Instruments, Austin, TX). Validation studies[23] confirmed that the linear variable differential transformer voltage output was linear with respect to the Schiøtz scale reading, where each scale reading is equivalent to 0.05 mm of plunger displacement.

The procedure was then repeated (after 10 minutes) on the contralateral eye, with patching of the already tested eye (to prevent corneal desiccation).

At present, the clinical measurement of uveoscleral outflow in humans is not possible;[24] thus, this value is generally calculated

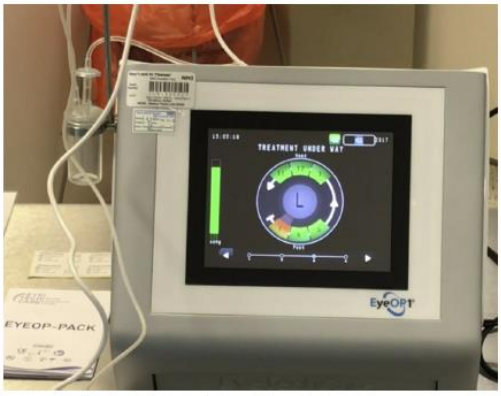

Figure 1. The console, transducer, and coupling cone.

from the Goldmann equation. Sit and McLaren[25] used a computerized venomanometry to measure episcleral venous pressure (EVP). They illustrated that EVP in normal subjects can vary between 6 and 10 mmHg. Therefore, we used this EVP range for our calculations. Uveoscleral outflow was calculated using a Goldmann equation (Equation 2) with an assumed EVP of 6 to 10 mmHg.

"*Ff*" is the rate of aqueous humor formation measured by fluorophotometry, "*C* "is the tonographic facility of outflow, "*Pi*" is the IOP, "*Pe*" is the EVP, and "*Fu*" is uveoscleral flow.

$$Ff = (Pi - Pe)C + Fu. \textit{Equation } 2$$

Therefore

$$Fu = Ff - C(Pi - Pe) \textit{ Equation } 3$$

High-Intensity Focused Ultrasound Treatment

The device consists of a console, transducer, and coupling device (Fig 1). Three different probe sizes are available to account for differences in ocular anatomy. The probe size is determined for each patient by optical biometry performed at baseline visit (measuring white-to-white diameter). A nomogram has been developed to facilitate white-to-white measurement to work out the probe size.

A coupling cone made of a polymer is placed on the eye (Fig 2). This is to ensure the centration and distance from the eye are maintained throughout the procedure. At the base of the probe, there is a suction device to create a low-level vacuum (225 mmHg) to stabilize the device on the eye. The 4-ml cavity that is created between the eye, cone, and treatment probe is filled with sterile saline solution at room temperature (BSS, Alcon Inc, Fort Worth, TX, or equivalent product). The 6 elliptical cylinder-shaped impacts are centered on an 11- to 13-mm diameter circle, depending on the ring diameter chosen, and spread over the eye circumference, while avoiding the nasal–temporal meridian (Fig 2). We used a second-generation probe. This differs from the original version in its broader active transducer area (0.2×4 mm^2 instead of 0.2×2.5 mm^2) and more precise temperature calibration of each single transducer. Other enhancements of the second-generation probe include optimized suction and centering on the eye globe, improved coupling of ultrasound due to removal of air bubbles in the liquid that could disturb the ultrasound beam, and optimized ergonomics and improved clip to attach the probe into the cone.

Figure 2. Close-up view of the coupling cone and transducer.

All HiFU procedures were performed by an experienced surgeon (K.S.L.) under peribulbar anesthesia (a mixture of lidocaine 2% and levobuprocaine 7.5%). Postoperatively, patients were treated topically with dexamethasone 0.1% preservative free 2 hourly for 2 weeks and then 4 times per day for 2 weeks. Hypotensive medications were stopped immediately postoperatively.

Postoperative Visits

Patients were reviewed at 1 week, 1 month, and 3 months postoperatively. The hypotensive topical drops may have been resumed at the discretion of the treating clinician. There was no washout at 1 week and 1 month postoperatively. However, all hypotensive treatments were stopped for 4 weeks before 3 months aqueous humor dynamic measurements (patients had a 2-week safety visit after commencing the washout). At the 2-week safety visit, patients with IOP >35 mmHg were treated with rescue medication and withdrawn from the study. Only 1 eye treated with HiFU (the worse eye affected by glaucoma/OHT) per participant was included in the data analysis.

Primary Outcome Measures

- IOP
- Facility of tonographic outflow (measured by digital Schiøtz tonography)
- Aqueous flow rate (measured by fluorophotometry)
- Uveoscleral outflow (calculated from the Goldmann's equation)

Table 1. Baseline Characteristics of Treated and Control Eyes

	Treated Eyes (n=30)	Control Eyes (n=13)	P Value	95% CI
Age, yrs	60.1±13	60.3±13	0.7	−8.7 to 9.1
Race				
Black	18	7	–	–
White	12	6	–	–
Diagnosis				
OHT	3	3	–	–
POAG	26	10	–	–
PG	1	0	–	–
Gender				
Female	14	7	–	–
Male	16	6	–	–
BCVA	54.1±5.1	56.5±3.2	0.09	−5.3 to 0.4
Central corneal thickness, µm	529±38	547±39	0.6	−8.5 to 44.0
Anterior chamber depth, mm	3.27±0.3	3.36±0.4	0.4	−0.1 to 0.3
Axial length, mm	24.1±1.1	24.1±0.9	0.7	−0.8 to 0.7
White-to-white, mm	11.9±0.3	11.8±0.32	0.9	−0.3 to 0.1
No. of medications	3.2±0.7 (median = 3)	2.8±1.0 (median = 3)	0.1	−0.1 to 0.9
MD, dB	−11.4±6.8 −25, 0.79 to −10.3 (median)	−3.7±5.9 −20, 1.18 to −2.61 (median)	**<0.001***	−11.7 to −3.6

BCVA = best-corrected visual acuity; CI = confidence interval; dB = decibels; MD = mean deviation; OHT = ocular hypertension; PG = pigment dispersion; POAG = primary open-angle glaucoma.
*Boldface indicates statistical significance.

These parameters were measured pretreatment (after 4 weeks of glaucoma treatment washout) and then at 3 months postsurgery (after 4 weeks of glaucoma medication treatment washout). Measures were taken from the operated eye and the contralateral nonoperated eye that was used as the control. At the 3-month visit, no patient had anterior chamber inflammation detectable with slit-lamp biomicroscopy. No quantitative assessment was performed.

Statistical Analyses

Histograms and 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 *t* test was 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. Linear regression analyses were used to determine the correlation of 1 parameter versus another parameter of aqueous humor dynamics. $P < 0.05$ was considered statistically significant (all analyses, SPSS 24.0; SPSS, Chicago, IL).

Sample Size Calculation

The sample size estimate was based on the results of paired measurements of 2 parameters (aqueous flow and facility of outflow) in a previous study done at the Mayo Clinic, Rochester, Minnesota, by the chief investigator (K.S.L.).[26] This study had a 90% chance of finding a 5% difference in IOP, 5.4% difference in aqueous flow, and 7.5% difference in outflow facility among medication groups, if these differences existed (n=30 subjects, α= 0.05, and ß=0.10).

Results

A total of 36 patients were invited to take part in this study. Three patients declined to participate, and 1 patient did not show up for the screening visit. One patient who had high IOP after washout during the safety visit was withdrawn from the study and had to have an urgent filtration surgery. One patient who had satisfactory IOP after washout was also excluded from the study because the surgical intervention was not required anymore.

Thirty eyes of 30 patients were included in the study. The study population included predominantly black (n = 18, 60%) and male (n = 16, 53%) patients. Baseline characteristics are shown in Table 1. Primary open-angle glaucoma was the main diagnosis. At the baseline study visit, the mean washout IOP was 31.7±5.3 mmHg with an average number of medications of 3.2±0.7 (median of 3).

Thirteen fellow eyes of the recruited patients who did not receive any surgical intervention during or before the study formed

Table 2. Baseline Aqueous Humor Dynamics of the 2 Groups

	Treated Eyes (n=30)	Control Eyes (n=13)	P Value	95% CI
Baseline IOP (mmHg)	31.7±5.3	28.2±5.1	0.9	−0.7 to 0.07
Baseline aqueous flow rate (µl/min)	2.08±0.7	2.06±0.4	0.5	−0.6 to 0.3
Baseline tonographic outflow facility (µl/min/mmHg)	0.14±0.09	0.12±0.09	0.6	−0.08 to 0.04
Baseline uveoscleral outflow (µl/min)	−0.87±2.17	−0.14±1.5	0.4	−0.5 to 2.2

CI = confidence interval; IOP = intraocular pressure.

Table 3. Summary of Treatment Failure Cases

Cases	Diagnosis	Age (Yrs)	Race	No. of Preoperative Medications (No Acetazolamide)	Prewashout IOP*	Postwashout IOP*	Postoperative IOP* at 1 Wk	No. of Medications at 1 Wk	Postoperative IOP* at 1 Mo	No. of Medications at 1 Mo
1	PG	40	White	4	28	40	33	2	46	3+ acetazolamide
2	POAG	72	White	2	26	37	13	0	29	4+ acetazolamide
3	POAG	74	Black	4	23	33	14	0	27	4+ acetazolamide
4	POAG	45	Black	3	25	30	10	0	45	3+ acetazolamide
5	POAG	53	Black	4	24	37	22	0	28	3

IOP = intraocular pressure; PG = pigment dispersion glaucoma; POAG = primary open-angle glaucoma.
*IOP was measured with a Goldmann applanation tonometer.

the control group. The baseline characteristics are shown in Table 1. The baseline aqueous humor dynamic measurements are shown in Table 2. There was no statistically significant difference in all the parameters between the treated and controlled groups.

High-Intensity Focused Ultrasound Treatment

High-intensity focused ultrasound treatment was performed in all 30 eyes. In all cases, 6 sectors were treated except 1 patient whose treatment was prematurely terminated by the operator so that only 4 sectors were treated because of discomfort felt by the patient. Five subjects were considered as treatment failure (despite maximal medical therapy post-HiFU treatment IOP remained suboptimal, and they had to have glaucoma filtration surgery after HiFU application). A summary of treatment failure cases is shown in Table 3.

Twenty-four patients completed 3-month aqueous humor dynamics measurements. One patient was lost to follow-up, and 5 patients had early treatment failure before 3 months washout and needed glaucoma filtration surgeries, and therefore withdrew from the study.

At the 3-month postoperative visit (only cases who did not need any further glaucoma surgical interventions were considered), the mean postwashout IOP was reduced by 16% (31.7±5.3 vs. 26.6±4.8 mmHg, $P = 0.004$), and aqueous flow rate was decreased by 15% (2.07±0.73 vs. 1.77±0.55 µl/min, $P = 0.05$) from baseline. Neither the tonographic outflow facility nor the uveoscleral outflow showed significant change from baseline. Table 4 illustrates primary outcome measures in the treatment group. The aqueous humor parameters remain unchanged at 3 months in the control group (Table 5).

By taking into account those 5 treatment failure eyes who were unable to undergo the 3 months washout visit, the proportion of eyes achieving IOP reduction of >20% at 3 months washout visit compared with the pretreatment washout IOP was 26.7% (n = 8); for IOP reduction of >30%, the proportion of eyes was 10% (n = 3). Overall, the HiFU procedure was well tolerated by patients. Table 6 summarizes the perioperative and postoperative complications.

Discussion

The current study investigated the aqueous humor dynamics effect of a cyclodestructive procedure and specifically HiFU in patients with uncontrolled open-angle glaucoma on maximum tolerated medical therapy, and this washout study involved a cycloablation. We demonstrated that there is a 20% risk of treatment failure (those who needed further glaucoma surgery intervention) within 1 month after a single HiFU treatment. Only 25 patients (80%) were able to undergo post-treatment washout measurements, and in these eyes, HiFU reduced IOP 3 months postoperatively by 16%, whereas aqueous flow decreased by 15% without any significant effect on tonographic outflow facility and uveoscleral outflow. Only 26.6% of eyes achieved >20% IOP reduction at 3 months compared with baseline.

Ciliary body treatment for glaucoma has been available for more than a century. The most obvious mechanism of

Table 4. Aqueous Humor Parameters Comparison before and after High-Intensity Focused Ultrasound Treatment

	Baseline (n=30)	3 Mos Postoperatively (n=24)	P Value	95% CI
IOP (mmHg)	31.7±5.3	26.6±4.8	**0.004***	1.3–6.4
Aqueous flow rate (µl/min)	2.07±0.73	1.77±0.55	**0.05†**	0.1–0.6
Tonography outflow facility (µl/min/mmHg)	0.14±0.09	0.15±0.13	0.6	−0.07 to 0.05
Uveoscleral outflow (µl/min)	−0.79±1.75	−0.53±1.76	0.6	−1.2-0.7

CI = confidence interval; IOP = intraocular pressure.
Boldface indicates statistical significance.
*Statistically significant.
†Wilcoxon signed-ranked test.

Table 5. Aqueous Humor Parameters of Control Group

	Baseline (n=13)	3 Mos Postoperatively (n=13)	P Value	95% CI
IOP (mmHg)	28.2±5.1	26.8±3.4	0.2	−0.7 to 3.5
Aqueous flow rate (μl/min)	2.26±0.69	2.22±0.70	0.4	−0.5 to 0.2
Tonography outflow facility (μl/min/mmHg)	0.13±0.08	0.13±0.08	0.3	−0.05 to 0.03
Uveoscleral outflow (μl/min)	−0.16±1.57	0.1±1.57	0.3	−0.84 to 0.3

CI = confidence interval; IOP = intraocular pressure.

action is the destruction of ciliary epithelium and subsequent reduction in aqueous humor production. Other possible adjunct mechanisms of action have been proposed, such as increased uveoscleral outflow[20] (external diode, micropulse, and HiFU) and flow-through sclera (HiFU).[21] Although various indirect methods of measurements have strengthened this belief,[21] there has never been any human aqueous humor dynamics study on the effect of ciliary body treatment.

Previous animal studies using HiFU in 18 rabbits' eyes reduced IOP by more than 50% four weeks after treatment,[19] whereas other clinical studies in humans achieved between 25.5% and 38% of IOP reduction.[18,27-30] However, none of these clinical studies performed washout from glaucoma medications before study measurements. The reason for the discrepancies in the extent of IOP reduction between previous studies and our results may be due to better glaucoma treatment compliance post-treatment and heterogeneity of glaucoma diagnoses in previous studies.

The most widely held view regarding the mechanism of IOP-lowering effect post-HiFU is through decreased aqueous production, which is consistent with our findings. However, there have been speculations as to whether HiFU may affect uveoscleral outflow and other aqueous humor dynamics parameters.[31] Mastropasqua et al[21] demonstrated anatomic alterations of sclera and conjunctiva 4 weeks after application of HiFU. They used these findings to contemplate that uveoscleral outflow enhancement may play a role in IOP reduction. However, the results from our aqueous humor dynamics measurement do not support this hypothesis. In fact, we did not find any statistically significant change in uveoscleral and trabecular outflow 3 months after the HiFU treatment, although earlier effects on other aqueous humor dynamics parameters such as uveoscleral outflow before our 3-month measurement cannot be ruled out by our study.

In the aforementioned histologic study of rabbits' eyes that underwent HiFU treatment, the authors[19] described coagulation necrosis lesions in the ciliary processes post-HiFU treatment. They observed the lesions were circumferentially distributed with the loss of the bi-stratified epithelium and edema and vascular congestion of the ciliary stroma without any histologic changes to the scleral tissue adjacent to the treated area. These findings appeared to support the aqueous humor dynamics findings from our study of reduced aqueous flow rate.

To explore if the aqueous flow rate change can fully account for the reduction in IOP at 3 months, we made some calculations using the Goldmann equation (Appendix 1, available at www.ophthalmologyglaucoma.org). If we assume that other aspects of aqueous dynamic parameters, such as tonographic outflow facility (C), uveoscleral outflow (*Fu*), and EVP (*Pe*), are not affected by HiFU and the mechanism of the IOP-lowering effect of HiFU is solely via the decreased aqueous flow rate (Ff) alone, then according to Goldmann's equation, a 16% reduction in IOP (Pi) at 3 months seen in our study should correspond to 15% decrease in aqueous flow rate (Ff), and this correlated well with our measured reduction of 12% in aqueous flow rate at 3 months. Because this largely accounts for the extent of IOP reduction, one can conclude that the reason for the IOP decrease after HiFU is likely to be caused by a decrease in aqueous flow rate alone (Appendix 1, available at www.ophthalmologyglaucoma.org).

Study Limitations

We did not perform any aqueous humor dynamics study measurements before the 3-month visit, and there may have been changes in the other aspects of aqueous humor dynamics parameters, such as uveoscleral outflow; however, aqueous flow measurements using fluorophotometry can be inaccurate in active uveitis. This is due to the presence of excessive protein in the anterior chamber, and the breakdown of the blood–aqueous barrier (during the early postoperative period after HiFU) can distort the assumptions about the standard diffusional loss of fluorescein during fluorophotometry.[32] Furthermore, the use of topical steroids may adversely affect the trabecular outflow.[33,34] We have chosen the 3-month time point as the first opportunity, after the cessation of topical steroid use and postoperative uveitis is resolved, to perform aqueous humor dynamics measurement. None of our patients had repeat HiFU treatment. This is in contrast to all previous studies in which patients had more than 1 application of HiFU treatment. Repeated treatment application may augment the effect of

Table 6. List of Adverse Events

Lens opacity	1
Scleral marks	4
Pain during the procedure*	4
Persistent uveitis	1
Punctate epithelial erosions	2
Hypotony	0
Visual loss (>2 Snellen lines)	1

*Led to incomplete treatment.

HiFU. However, because our primary interest was in the aqueous humor dynamics effects of HiFU treatment, repeated treatment would have caused further intraocular inflammation, thus delaying the aqueous humor dynamics measurement further in some patients. The result of our study may not be generalizable to other types of cyclodestructive treatments such as cyclodiode because there may be a different mechanism of action due to laser light scatter and potentially wider treatment area. Finally, because there was no quantitative measure of anterior chamber flare/inflammation in this study, there may have been some subclinical inflammation at the 3-month visit.

In conclusion, our clinical study shows the aqueous humor dynamic changes after HiFU treatment. We confirmed that at 3 months post-HiFU treatment, reduction in aqueous production is the only aqueous dynamic parameter that could have accounted for the decrease in IOP.

Acknowledgments. The authors thank Stephanie Jones for administration and managerial support for the study.

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Footnotes and Financial Disclosures

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[1] St. Thomas' Hospital, London, United Kingdom.

[2] King's College London, London, United Kingdom.

[3] Imperial College London, London, United Kingdom.

Presented at: the Association of Research in Vision and Ophthalmology, Honolulu, Hawaii, April 29, to May 3, 2018.

Financial Disclosure(s):
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K.S.L.: Research grant — New World Medical, Iridex, BVI, EyeTechCare, Ellex; Investigator — Ivantis, Allergan, PGI, Rheon; Advisory board — Glaukos, Alcon; Consultant — iStar; Support — National Institute for Health Research Biomedical Research Centre at Guy's and St Thomas NHS Foundation Trust (GSTT).

Funded by a grant from the EyeTech Company (funding number 06/03/2015) however, the company did not have any influence on the conduct of the study data collection, statistical analysis, or manuscript preparation.

HUMAN SUBJECTS: Human subjects were included in this study. The human ethics committees at the National Health Service approved the study. All research adhered to the tenets of the Declaration of Helsinki. All participants provided informed consent.

No animal subjects were used in this study.

Author Contributions:

Conception and design: Alaghband, Lim

Data collection: Alaghband, Galvis, Ramirez, Madekurozwa, Chu, Overby, Lim

Analysis and interpretation: Alaghband, Overby, Lim, Ramirez

Obtained funding: Alaghband, Lim

Overall responsibility: Alaghband, Overby, Lim

Abbreviations and Acronyms:
EVP = episcleral venous pressure; **HiFU** = high-intensity focused ultrasound; **IOP** = intraocular pressure; **OHT** = ocular hypertension; **TOF** = tonographic outflow facility.

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The ethical approval letter for the effect of phacoemulsification on outflow facility

NHS

National Research Ethics Service

St Thomas' Hospital Research Ethics Committee

South London REC Office 3
Ethics Committee Office
Governors' Hall Suite,
Ground Floor South Wing
St Thomas' Hospital
London
SE1 7EH

Telephone: 020 7188 2257
Facsimile: 020 7188 2258

16 November 2009

Mr K S Lim
Consultant Ophthalmologist
Guy's and St. Thomas' NHS Foundation Trust
Eye Department,
St. Thomas' Hospital
Westminister Bridge Road,
London, SE1 7EH

Dear Mr Lim

Study Title:	**Effect of Phacoemulsification on outflow facility in eyes with and without primary open angle glaucoma: comparative clinical study**
REC reference number:	**09/H0802/103**
Protocol number:	**1.1**

The Research Ethics Committee reviewed the above application at the meeting held on 09 November 2009.

Ethical opinion

The members of the Committee present gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

Ethical review of research sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

For NHS research sites only, management permission for research ("R&D approval") should be obtained from the relevant care organisation(s) in accordance with NHS research governance arrangements. Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where the only involvement of the NHS organisation is as a Participant Identification Centre, management permission for research is not required but the R&D office should be notified of the study. Guidance should be sought from the R&D office where necessary.

Sponsors are not required to notify the Committee of approvals from host organisations.

It is responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The documents reviewed and approved at the meeting were:

Document	*Version*	*Date*
REC application		17 September 2009
Protocol	1.1	03 September 2009
Investigator CV		03 September 2009
Letter of invitation to participant	1.1	03 September 2009
Response to Request for Further Information		
Participant Information Sheet	1.2	23 October 2009
Participant Consent Form	1.2	23 October 2009
GP/Consultant Information Sheets	1.2	23 October 2009

Membership of the Committee

The members of the Ethics Committee who were present at the meeting are listed on the attached sheet.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Now that you have completed the application process please visit the National Research Ethics Service website > After Review

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

We would also like to inform you that we consult regularly with stakeholders to improve our service. If you would like to join our Reference Group please email referencegroup@nres.npsa.nhs.uk.

09/H0802/103	**Please quote this number on all correspondence**

With the Committee's best wishes for the success of this project

Yours sincerely

Dr Robert Carr
Chair

Email: stella.hirsch@gstt.sthames.nhs.uk

Enclosures: *"After ethical review – guidance for researchers"*

Copy to: *Dr P Alaghband*
Karen Ignatian

The ethical approval letter and IGA/RCOphth grant approval letter for the aqueous humour dynamics in uveitic eyes

NHS
Health Research Authority
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Bristol Research Ethics Committee Centre
Whitefriars
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18 September 2013

Mr K. Sheng Lim
Consultant Ophthalmic Surgeon
Guy's & St Thomas' NHS Foundation Trust
St Thomas' Hospital
Westminster Bridge Road
London
SE1 7EH

Dear Mr Lim

Study title:	**The contribution of altered aqueous dynamics in the development of raised intraocular pressure in patients with uveitis**
REC reference:	**13/LO/1249**
IRAS project ID:	**136787**

Thank you for your letter of 29 August 2013, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

We plan to publish your research summary wording for the above study on the NRES website, together with your contact details, unless you expressly withhold permission to do so. Publication will be no earlier than three months from the date of this favourable opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to withhold permission to publish, please contact the Co-ordinator Mrs Vicky Canfield-Duthie, nrescommittee.london-bromley@nhs.net.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
GP/Consultant Information Sheets	2.0	03 July 2013
Investigator CV		
Participant Consent Form: Uveitic Patients	3.0	
Participant Consent Form: Healthy Controls	1.0	
Participant Information Sheet	2.0	03 July 2013
Participant Information Sheet: Uveitic Patients	3.0	28 August 2013
Participant Information Sheet: Healthy Controls	1.0	28 August 2013
Protocol	2.0	26 July 2013

REC application		
Response to Request for Further Information		29 August 2013

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

Further information is available at National Research Ethics Service website > After Review

13/LO/1249	**Please quote this number on all correspondence**

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

With the Committee's best wishes for the success of this project.

Yours sincerely

Ms Carol Jones
Chair

Email:nrescommittee.london-bromley@nhs.net

AGREEMENT FOR RESEARCH GRANT
FROM THE INTERNATIONAL GLAUCOMA ASSOCIATION

THIS AGREEMENT is made the 16 day of JANUARY 2014 BETWEEN

(1) The present trustees of the charity called the International Glaucoma Association of Woodcote House, 15 Highpoint Business Village, Henwood, Ashford, Kent TN24 8DH ("the Trust")

(2) Guy's and St Thomas's NHS Foundation Trust, of St Thomas' Hospital, Westminster Bridge Road, Westminster Bridge Road, London, SE1 7EH ("the Recipient")

TITLE OF PROJECT/APPLICATION:

The contribution of Altered Aqueous Dynamics in the Development of Raised Intraocular Pressure in Patients with Uveitis (the "Project")

NOW IT IS AGREED as follows:

1.0 Introduction

1.1 The principal object of the Trust is to prevent blindness through awareness education and research

1.2 The Researcher on behalf of the Recipient has satisfied the Trust that they have the knowledge, facilities and expertise required to carry out useful research in this field

1.3 The Trust has agreed to pay the Grant Monies to the Recipient for the Project on the terms and conditions set out in this Agreement

1.4 It is intended that two of the present trustees of the Trust named above will execute this Agreement in the names and on behalf of them all pursuant to a resolution of the Trust under Section 82 of the Charities Act 1993

1.5 The definitions and interpretation which appear at the end of this agreement shall apply to the provisions hereof

1.6 Mr Sheng Lim, an employee of the Recipient will be Chief Investigator for the Project ("the Researcher")

2. The Trust's Obligations

2.1 The Trust undertakes to pay the Grant Monies in accordance with the Payment Provisions on receipt of a proper invoice from the Recipient

2.2 The Grant will be paid in line with the following schedule upon receipt of the proper invoice plus a short written report of progress (excepting the initial payment):

1st Instalment	£ 6,626.50
2nd Instalment	£ 6,626.50
3rd and final Instalment	£ 1,000.00

Total Grant - £14,253.00

2.3 The final £1000.00 will only be paid upon receipt of a final report approved by the Chair of the IGA Grants Committee and invoice at the conclusion of the Project

2.4 The Trust undertakes (without being in any way committed to any increase in its support) to consider a pro rata increase in the Grant Monies on notification from the Recipient of any nationally agreed pay award for staff of the Recipient involved in the Project or for any other reasonable cause

3.0 Recipient's Obligations

3.1 The Researcher on behalf of the Recipient shall undertake to carry out the Project in accordance with the Project Plans (subject to any such variation as may be agreed in writing between the parties to this Agreement) and should there by any significant change

in research personnel, it will require the approval of the Chairman of the IGA Grants Committee.

3.2 The Researcher on behalf of the Recipient undertakes to inform the Trust promptly if for any reason they are unable or unlikely to fulfil the timetable set out in any of the Project Plans

3.3 The Researcher on behalf of the Recipient undertakes to report progress on the Project to the Trust annually by producing to the Trust without being requested so to do, a written report for each year of the Project within three months of the end of that year and arranging at the request of the Trust for the Researcher to attend for an interview with officers of the Trust at a time and place in the United Kingdom which is convenient to such officers

3.4 The Researcher on behalf of the Recipient further agree to provide to the Trust at any time on request (such requests not to be made more than four times in any year) full information about the progress to date of the Project and/or a short written report suitable for submission by the Trust to other grant-making bodies in support of applications for funds for the Project or for any similar project

3.5 The Recipient undertakes to ensure that the Project is carried out in accordance with best practice in order to avoid damage, loss or injury to participants or property and the Recipient undertakes to take all reasonable precautions to safeguard the health and safety of those participants involved in the Project. The Trust accepts no liability for any accident, injury or loss sustained by any person as a result of and/or in the course of the Project

3.6 The Recipient undertakes that it has obtained and will maintain in force for the duration of the Project and for a period of 5 years thereafter, public and professional indemnity insurance at a level appropriate to the risk involved

3.7 At the conclusion of the Project the Researcher on behalf of the Recipient shall arrange for all useful results thereof to be published and may arrange for publication of interim results or other reports concerning the Project at any time and in respect thereof:-

3.7.1 the Researcher on behalf of the Recipient shall use their best endeavours to ensure that the publishers shall include an acknowledgment of the Trust's having contributed to the Project

3.7.2 the Researcher on behalf of the Recipient shall give advance written details to the Trust of each proposed publication including, where relevant, the name and address of the journal or broadcasting station concerned, the proposed date of publication and a summary or abstract of the content (and the Trust will not make public any information so provided except with the prior agreement of the Recipient)

4.0 Intellectual Property

4.1 The Intellectual Property Rights shall belong to the Recipient

4.2 The Recipient undertakes:-

4.2.1 to take all reasonable steps to register protect and exploit all Intellectual Property Rights

4.2.2 to pay out of the net proceeds of any such exploitation such reasonable awards to individual contributors as may be agreed with the Trust

4.2.3 to pay to the Trust a percentage of the remaining proceeds of such exploitation such percentage to be agreed at the conclusion of the Project as fairly representing the Trust's contribution to the Intellectual Property.

4.2.4 to provide to the Trust an annual statement of account in such form as may from time to time be approved by the Trust of the results of exploiting any of the Intellectual Property Rights

4.3 The Recipient hereby agrees and it is a condition of this Agreement to provide the Trust with a non-exclusive, non-sublicensable licence for the Trust's use of the Intellectual Property Rights for non-commercial research purposes only. The Trust will (subject to any such reasonable embargo as may for a time be placed by the Recipient upon publication of any details of the Project) have full right without payment or further permission to use (in furtherance only of its non-commercial charitable work and charitable objectives) of all Intellectual Property Rights.

5.0 Variation

These terms may be varied at any time by agreement in writing between the Officers without prejudice to the validity of any acts or events taking place before such variation under these terms

6.0 Termination by the Recipient

6.1 The Recipient may terminate this Agreement at any time by giving four weeks' notice in writing to the Trust that no further Grant Monies are required for the Project without prejudice nevertheless to the validity of any acts or events taking place before such notice is received by the Trust

6.2 On such termination the Researcher on behalf of the Recipient shall provide a final report to the Trust and an account of the Grant Monies received and spent under the terms of this Agreement

6.3 The Recipient shall repay any unused Grant Monies to the Trust within four weeks of the date of such termination

7.0 Termination by the Trust

7.1 The Trust may terminate this Agreement by giving three months' notice to the Recipient that no further Grant Monies shall be paid provided that such notice may only be given on one or more of the following grounds (which shall be specified in the said notice) namely:-

7.1.1 breach by the Recipient of any of the terms of this Agreement (including any variation of them made under clause 5 above) or

7.1.2 the failure of the Recipient (whether actual or reasonably anticipated) to meet the objectives of the Project for the then current period of the Project Plans or

7.1.3 that satisfactory arrangements for funding the Project from other sources have been made or

7.1.4 that the Trust will not have sufficient funds after the expiry of the notice to continue to fund the Project

7.2 In the event of termination by the Trust for either of the reasons set out in Clauses 7.1.1 or 7.1.2 then the Recipient shall repay any uncommitted Grant Monies to the Trust within four weeks of the date of such termination

8.0 Communication

8.1 Communications concerning or under the terms of this Agreement shall be made between the Officers

8.2 Any notice to be served on any party by any other shall be in writing and shall be sent either:-

8.2.1 by prepaid recorded delivery first class post in which case it shall be deemed to have been received on the second Working Day after posting or

8.2.2 by facsimile transfer to the correct number of the receiving party in which case it shall be deemed to have been received on the same day as it was transmitted or the next Working Day if transmitted on a day other than a Working Day or after 3:00 p.m.

9.0 Miscellaneous

9.1 This Agreement shall constitute the entire agreement and understanding between the parties with respect to all matters which are referred to herein and shall supersede any previous agreement(s) between the parties in relations to such matters

9.2 If any term or provision in this Agreement shall in whole or in part be held to any extent to be illegal or unenforceable under any enactment or rule of law that term or provision or part thereof shall to that extent be deemed not to form part of this Agreement and the enforceability of the remainder of this Agreement shall not be affected

9.3 This agreement is made under and shall be governed by English Law

AS WITNESS the hands of the parties hereto the day and year first before written

FIRST SCHEDULE
(description of whole project)

SECOND SCHEDULE
(details of grant aid)

THIRD SCHEDULE
(details of project plans)

FOURTH SCHEDULE
(names or descriptions of officers)

FIFTH SCHEDULE
(shares of ownership of Intellectual Property Rights (if applicable))

Signatures of the parties

IGA Chair of Trustees .....

On behalf of the Recipient Organisation .....

IGA Honorary Treasurer .....

On behalf of the Researcher
Mr Sheng Lim .....

Definitions and interpretation

1 In this Agreement the following expressions shall have the following meanings:-

1.1 "The Project" shall mean the research project details of which are given in the first schedule hereto

1.2 "The Grant Monies" shall mean the monies specified in the second schedule hereto

1.3 "The Payment Provisions" shall mean the provisions as to the timing and method of payment of the Grant Monies which are also set out in the second schedule hereto

1.4 "The Project Plans" shall mean such annual or other plans for the carrying out of the Project as are detailed in the third schedule hereto

1.5 "The Intellectual Property Rights" means all intellectual property rights of the Recipient deriving from the Project generated under this Agreement including without limitation patents trade marks and/or service marks (whether registered or unregistered) registered designs unregistered designs and copyrights (and any applications for any of the same) owned by the Grantees

1.6 'The "Researcher" shall mean Mr Sheng Lim in the Department of Ophthalmology, St. Thomas's Hospital, South Wing, Westminster Bridge Road, London SE1 7EH'

1.7 "The Officers" shall mean the officers authorised by the Trust and the Recipient respectively (whose names or descriptions are set out in the fourth schedule hereto) and the Researcher personally

1.8 "Working Days" shall mean Monday to Friday inclusive save for any days which are public or statutory holidays

2. Words denoting the singular number only include the plural and vice versa

3. Words denoting any gender include all genders and words denoting persons include firms and corporations and vice versa

4. Unless the context otherwise requires reference to any clause sub-clause paragraph or schedule is to a clause sub-clause paragraph or schedule (as the case may be) of or to this Agreement

5. The headings in this document are inserted for convenience only and shall not affect the construction or interpretation of this Agreement

6. Reference to any statute or statutory provision includes a reference to that statute or provision as from time to time amended extended re-enacted or consolidated and to all statutory instruments or orders made under it

7. Reference to any schedule hereto may mean either a schedule forming part of this agreement or a separate schedule attached to this agreement and initialled by the parties hereto

8. Any obligation upon or undertaking given by more than one party to this agreement shall be the joint and several liability of them

The ethical approval letter for the effect of High intensity focused ultrasound on aqueous humour dynamics

NHS
Health Research Authority
London - Camden & Kings Cross Research Ethics Committee
Room 001
Jarrow Business Centre
Rolling Mill Road
Jarrow
Tyne & Wear
NE32 3DT

Telephone: 0191 428 3444

29 February 2016

Mr. K Sheng Lim
Consultant Ophthalmologist
Guy's & St Thomas' NHS Foundation Trust
Department of Ophthalmology, Block 7, South Wing,
St Thomas' Hospital
Westminster Bridge Road
London
SE1 7EH

Dear Mr. Lim

Study title:	**The Effect of Glaucoma Surgery on Aqueous Dynamics in Patients with Glaucoma or Ocular Hypertension: An Observational Study**
REC reference:	**15/LO/1809**
IRAS project ID:	**163050**

Thank you for your letter of 23 February 2016, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair with the Lead Reviewer.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to make a request to postpone publication, please contact the REC Assistant, Miss Kirstie Penman at nrescommittee.london-camdenandkingscross@nhs.net.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a **favourable** ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation

as revised, subject to the conditions specified below.

Conditions of the favourable opinion

The REC favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements. Each NHS organisation must confirm through the signing of agreements and/or other documents that it has given permission for the research to proceed (except where explicitly specified otherwise).

Guidance on applying for NHS permission for research is available in the Integrated Research Application System, www.hra.nhs.uk or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ('Participant Identification Centre'), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of management permissions from host organisations

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database within 6 weeks of recruitment of the first participant (for medical device studies, within the timeline determined by the current registration and publication trees).

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to contest the need for registration they should contact Catherine Blewett (catherineblewett@nhs.net), the HRA does not, however, expect exceptions to be made. Guidance on where to register is provided within IRAS.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see 'Conditions of the favourable opinion' above).

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Covering letter on headed paper [Covering letter_Aqueous Dynamics Study]		29 September 2015
GP/consultant information sheets or letters [Aqueous Dynamics GP letter]	1	16 August 2015
Letter from funder [Letter from Dietrich Wolf, Eye Care Tech]		07 July 2015
Other [Amended Protocol (V2, 18/01/2016) in track changes format]	2	18 January 2016
Other [Response to provisional opinion]		22 February 2016
Participant consent form [Aqueous Dynamics CF]	2	18 January 2016
Participant consent form [Aqueous Dynamics CF [Large text]]	2	18 January 2016
Participant information sheet (PIS) [Aqueous Dynamics PIS]	2	18 January 2016
Participant information sheet (PIS) [Aqueous Dynamics PIS [Version 2 in Track Changes format]]	2	18 January 2016
Participant information sheet (PIS) [Aqueous Dynamics PIS [Large text]]	2	18 January 2016
REC Application Form [REC_Form_01102015]		01 October 2015
Research protocol or project proposal [Aqueous Dynamics and Glaucoma Surgeries Study Protocol]	2	18 January 2016
Summary CV for Chief Investigator (CI) [CV Sheng Lim]		
Summary CV for student [P.Alaghband short CV 2015]		
Summary CV for supervisor (student research) [CV Chris Hammond]		

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document *'After ethical review – guidance for researchers'* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website:
http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/

HRA Training

We are pleased to welcome researchers and R&D staff at our training days – see details at http://www.hra.nhs.uk/hra-training/

15/LO/1809	**Please quote this number on all correspondence**

With the Committee's best wishes for the success of this project.

Yours sincerely
pp

Mrs Rosie Glazebrook
Chair

Email: nrescommittee.london-camdenandkingscross@nhs.net

Enclosures: "After ethical review – guidance for researchers" *[SL-AR2]*

Copy to: *Elizabeth Bruna, Guy's and St Thomas' NHS Foundation Trust*

Jennifer Boston, Guy's and St Thomas' NHS Foundation Trust

The funding agreement letter for the effect of HiFU on aqueous humour dynamics study

EYE TECH CARE | SOUND GLAUCOMA CARE

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www.EYE TECH CARE.com

Dr K. Sheng Lim
Consultant Ophthalmologist
St Thomas' Hospital, London

Rillieux La Pape, July 07, 2015

OBJECT: LETTER OF AGREEMENT

EYE TECH CARE and its affiliates frequently receive requests from clinicians / scientists to provide financial support for their research projects, with the majority of these investigations to be conducted with EYE TECH CARE products. EYE TECH CARE is in principle interested to support such research projects as they enhance the knowledge in research areas relevant to EYE TECH CARE and further generate company independent scientific data. The decision whether financial support is granted is based on the scientific/educational value of the study as assessed by the Investigator Driven Study Evaluation Committee (IDSEC)

We can confirm that an application for funding was received from Chief Investigator: Dr K. Sheng Lim, Consultant Ophthalmologist, St Thomas' Hospital, London on 06/30/2015. The proposal was to conduct a Aqueous Dynamic Effect of Glaucoma Surgeries.

The application plus supporting material was considered by the EYE TECH CARE / Dr Sheng LIM at a meeting on 03/10/2015 (and subsequent correspondences). We are pleased to confirm that a sum of £25,632 has been awarded for the project in accordance with the established protocol (Appendix 1) and the additional elements (Appendix 2).

EYE TECH CARE

Dietrich WOLF

Chief Executive Officer

Date: 07/09/2015

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APPENDIX 1 : PROTOCOL

Protocol version : *"Aqueous Dynamics Study Protocol 20_04_2015 on GCP Non CTIMP Protocol Template V4 Jan 2014"* (Attached)

APPENDIX 2: ADDITIONAL ELEMENTS

- **Number of patients enrolled :** 30

- **Inclusion/exclusion criteria** : POAG regardless of previous surgery or laser treatment (except SLT>6 months) with IOP baseline at 21-28mmHg before wash out and 32mmHg after wash-out.

- **Visual acuity measurements that need to be performed:** Best Corrected Distance Visual Acuity (BCDVA) , Non Corrected Distance Visual Acuity (NCDVA) , Best Corrected Near Visual Acuity (BCNVA)