ANGLIA RUSKIN UNIVERSITY
Faculty of Medical Science

# Obstructive Sleep Apnoea in people with Primary Open-angle Glaucoma: prevalence, associations and the impact of Continuous Positive Airway Pressure on Intraocular Pressure

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# Abstract

**Introduction**: Obstructive sleep apnoea (OSA) has been proposed as a contributing factor in the development and progression of primary open-angle glaucoma (POAG) but there is insufficient evidence to confirm or refute this hypothesis. The prevalence of OSA among people with POAG is unknown. To complicate the picture further, concerns have been raised that continuous positive airway pressure (CPAP) increases nocturnal intraocular pressure (IOP), currently the only treatable risk factor for POAG.

**Objectives:** The objective of this thesis was to assess whether the prevalence of OSA among patients with POAG is different from that in people without glaucoma and to examine for associations between apnoea-hypopnea index (AHI) and markers of functional and structural changes in POAG. In addition, the thesis examined whether POAG patients with untreated OSA have faster rates of retinal nerve fibre layer (RNFL) loss than those without OSA. The impact of CPAP on IOP and ocular microvasculature were investigated.

**Methods**: In order to examine the thesis' questions, studies with the following methodology were conducted: a cross-sectional observational study with a control group, a retrospective longitudinal data analysis, a prospective longitudinal study examining the effect of intervention and a physiological experiment.

**Results**: Based on data from 235 POAG patients and 160 controls the prevalence of OSA was 58% and 54%, respectively. After statistical matching, the proportion of participants diagnosed with OSA was not different between the groups (p=0.91). The AHI was not associated with the severity of visual field defect or RNFL thinning after adjustment for confounders in a cross-sectional study but, in a longitudinal study, people with OSA had almost twice faster rates of RNFL loss than those without OSA (-1.1±1.5 μm/year vs -0.6 ±1 μm/year, respectively, p=0.013) and this was independent of other risk factors. CPAP significantly raised nocturnal IOP in people with (+2.2±2.2mmHg) and without POAG (+3.2±2.2mmHg) but a diurnal application of CPAP at different levels in awake subjects was not associated with IOP elevation. A short-term treatment with CPAP did not alter the optic disc and retinal microvasculature.

**Conclusions:** The burden of OSA among POAG patients is not higher than in people without glaucoma but OSA may be an independent progression risk factor in those with established POAG. CPAP raises IOP and the long-term impact of this on POAG progression should be investigated in future studies.



**Key words:** obstructive sleep apnoea, primary open-angle glaucoma

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# Glossary of Abbreviations

AHI- apnoea-hypopnoea index

ANP- atrial natriuretic peptide

ARMD-age-related macular degeneration

AUC ROC- area under the receiver operating characteristic curve

BMI-body mass index

BRVO-branch retinal vein occlusion

CCT-central corneal thickness

CLS-contact lens sensor

CPAP-continuous positive airway pressure

CRVO- central retinal vein occlusion

CSA-central sleep apnoea

CSF-cerebro-spinal fluid

CVA-cerebrovascular accident

CVS-cardiovascular system

DBP-diastolic blood pressure

eCRF-electronic Case Report Form

eGSS-enhanced Glaucoma Severity Staging system

EDS-excessive daytime sleepiness

ESS- Epworth Sleepiness Scale

EVP-episcleral venous pressure

FEV1- forced expiratory volume in one second

FVC- forced vital capacity

GEE- generalised estimating equations

HFA- Humphrey Field Analyser

HTG- high tension glaucoma

HR- heart rate

ICD- international classification of diseases

IOP-intraocular pressure

IQR-interquartile range

MD-mean deviation

MeanSpO2-mean oxygen saturation

MinSpO2-minimum oxygen saturation

NS- not statistically significant

NTG-normal tension glaucoma

OCT-ocular coherence tomography

OCT-A-ocular coherence tomography angiography

OHT-ocular hypertension

ODI-oxygen desaturation index

ONH- optic nerve head

OPP- ocular perfusion pressure

OSA-obstructive sleep apnoea

OSAS-obstructive sleep apnoea syndrome

PEEP-positive end-expiratory pressure

PIS-participant information sheet

PSD- pattern standard deviation

PSG-polysomnography

POAG-primary open-angle glaucoma

RCT-randomised controlled trial

REM- rapid eye movement (sleep)

RGC-retinal ganglion cell

RNFL-retinal nerve fibre layer

ROS- radical oxygen species

rPG- respiratory polygrapy

RSSC- Respiratory Support and Sleep Centre

SBP- systolic blood pressure

SDC- Sleep Disturbance Clinic

SDB-sleep-disordered breathing

SD-standard deviation

SD-OCT-Spectral Domain Ocular Coherence Tomography

SER-Spherical Equivalent Refraction

SEM- standard error of mean

SLE-selective laser trabeculoplasty

SpO2-oxygen saturation

TNFα- tumour necrosis factor alpha

T90-time spent with oxygen saturation less than 90%

VD-vessel density

VF-visual field

VFI-visual field index

# List of Supporting Publications and Presentations

The work reported in Chapter 3 has been published in part in the Journal of Glaucoma: *Wozniak D, Bourne R, Peretz G, Kean J, Willshire C, Harun S, Villar S, Chiu YD, Smith I. Obstructive Sleep Apnea in Patients With Primary-open Angle Glaucoma: No Role for a Screening Program. J Glaucoma. 2019 Aug;28(8):668-675.*

The main results of Chapters 3, 4, 5 and 6 have been presented at the following conferences:

- British Thoracic Society (BTS) Winter Meeting, London, December 2018 and European Respiratory Society (ERS) congress, Paris, September 2018 (combined oral and poster presentations); *Wozniak D, Schneiders M, Bourne R, Smith I.CPAP related intraocular pressure increase at night in people with and without glaucoma.*
- British Thoracic Society (BTS) Winter Meeting, London, December 2018 and Oxford Ophthalmological Congress, July 2018 (oral and poster presentations); *Wozniak D, Ratwatte M, Bourne R, Smith I. Is Obstructive Sleep Apnoea a Risk Factor for Reduction of Retinal Nerve Fibre Layer Thickness in Primary Open-Angle Glaucoma?*
- European Respiratory Society (ERS) congress, Paris, September 2018 (poster presentation); *Wozniak D, Schneiders M, Bourne R, Smith I. Does intraocular pressure change in response to CPAP applied in wakefulness?*
- European Respiratory Society (ERS) congress, Paris, September 2018 and Association for Research in Vision and Ophthalmology (ARVO) congress, Honolulu, Hawaii, May 2018 and European Glaucoma Society (EGS) conference, Florence, Italy, May 2018 (poster presentations); *Wozniak D, Kean J, Peretz G, Harun S, Willshire C, Villar S, Smith I, Bourne R. Prevalence of Obstructive Sleep Apnoea in Glaucoma: the POSAG Study.*
- East Anglia Thoracic Society (EATS) meeting, May 2018 (oral presentation); *Wozniak D. CPAP and Intraocular Pressure.*
- Oxford 2nd Annual Meeting. The Effect of Ocular Disease on the Sleep/Wake Cycle, September 2016 (oral presentation); *Wozniak D. The effects of positive airway pressure on the eyes.*

# Chapter 1: Introduction

*1.1 Overview and motivation.*

This thesis examines several aspects of an intriguing and complex relationship that has been proposed between obstructive sleep apnoea (OSA) and primary-open angle glaucoma (POAG). Both conditions are common and each has been studied extensively over the last few decades by sleep specialists and ophthalmologists respectively. Much has been learnt about them separately, much is still unknown. Interdisciplinary research into the link between OSA and POAG has resulted in interesting hypotheses but also conflicting and often confusing findings. In many areas the existing evidence is insufficient to draw any conclusions.

The overarching goals of this thesis are to produce good quality evidence for questions that have been asked but not answered, challenge beliefs that may be lacking support in solid data, explore the usefulness of new technologies and form new hypotheses. Specifically, this thesis examines: the prevalence of OSA among people with POAG, the hypothesised associations between OSA and POAG severity markers, the role of OSA in POAG progression, the impact of continuous positive airway pressure (CPAP) on intraocular pressure and on the novel angiographic indicators of retinal microvasculature. The body of work consists of: a large cross-sectional observational study, a retrospective longitudinal analysis, a prospective longitudinal study examining the effect of intervention and a physiological experiment.

It is my hope that the work presented in this thesis will further the current knowledge on this puzzling interdisciplinary problem.

*1.2 POAG*

*1.2.1 What is Glaucoma?*

The word “glaucoma” originates from the ancient Greek word *glaucosis*, meaning light blue or blue-green hue. During the Hippocratic period it was probably used to describe the appearance of the pupil which was clouded as a result of media opacity (1). Today, the term “glaucoma” has a different meaning and encompasses a group of progressive diseases which cause degeneration of the optic nerve through the damage of the retinal ganglion cells (RGCs). These neurons are part of the central nervous system and play an essential role in signal transmission from the photoreceptors to the vision centres in the brain. Their cell bodies are located in the inner part of the retina and their axons form the optic nerve. The damage to the RGCs is irreversible and in a large proportion of patients leads to impaired vision, and in some, causes blindness. In fact, glaucoma is one of the leading causes of irreversible blindness worldwide. It has been projected that

79.6 million people will be affected by glaucoma by 2020 (2). Of those, 4.5 million will have moderate to severe vision loss and 3.2 million will be bilaterally blind (3). More than half of people with glaucoma are not aware that they have it as the disease often remains asymptomatic until the advanced stages (2).

There are different types of glaucoma, each with their own specific features and aetiologies. The most common form of glaucoma is POAG which is defined by the glaucomatous appearance of the optic disc in the presence of open anterior chamber angles. In the United Kingdom it is diagnosed in around 2% of people over 40 years of age (4). In contrast, angle-closure glaucoma is characterised by occlusion of the angle between the peripheral cornea and the iris where the eye drainage system is located and it is approximately five times less common (5). Glaucoma can be also caused by underlying factors such as blockage of the angle by exfoliated material from ocular structures (exfoliation glaucoma) or pigment granules (pigmentary glaucoma), formation of abnormal blood vessels on the iris (neovascular glaucoma) or inadequate development of ocular drainage channels (congenital glaucoma). Certain medications such as systemic corticosteroids can also lead to the development of glaucoma (6). However, these secondary forms of glaucoma are far less prevalent.

### *1.2.2 How is POAG diagnosed and monitored?*

In healthy eyes, the axons of RGCs converge at the optic disc creating the neuroretinal rim which surrounds the cup, a central shallow pit with no nerve fibres. A progressive loss of RGCs and their axons which occurs in POAG leads to characteristic changes in the appearance of the optic nerve head (ONH); the cup enlarges in diameter until it takes up the area of the optic disc. In addition, as a consequence of mechanical strain from intraocular pressure (IOP), the lamina cribrosa (a collagenous structure which provides mechanical support to the axon fibres) is displaced posteriorly and thinned which results in deepening of the cup (7). Also, the retinal nerve fibre layer (RNFL) surrounding the disc becomes thinner. These changes can be observed during ophthalmic examination of the optic disc with a slit lamp biomicroscope. This examination is crucial in POAG assessment however because of its subjective nature and a wide variation of the optic disc appearance in the healthy population there is a large inter-observer variability particularly in identification of early damage when the changes are subtle and more difficult to detect. The loss of RGC axons can also be objectively evaluated with several available imaging techniques. Of these, Ocular Coherence Tomography (OCT) has become the most popular as it provides precise, automated and reproducible measurement of RNFL thickness and thus is particularly useful in quantitative monitoring of progression. Detection of the structural changes is essential as it informs the early

diagnosis of POAG and allows for initiation of treatment before irreversible changes in vision develop. It is estimated that as much as 50% of RGCs are lost before functional defects can be demonstrated on standard visual field test (8,9). Nevertheless, it is the vision loss that matters most for the patient and it is important to make the connection between pathophysiological changes and patient related outcomes. For this reason, white-on-white automated perimetry has been used for decades and remains ubiquitous in glaucoma clinics. It employs white light stimuli with varying brightness on a white background in order to identify a minimum detection threshold at different locations in the visual field (VF). Peripheral vision usually declines first in glaucoma and central vision is preserved until the late stages of the disease. The typical pattern consist of superonasal followed by inferonasal field defects which correspond to inferotemporal followed by superotemporal RNFL loss (6). Perimetry relies on a patient responding to the stimulus and as such the output can be impacted by a learning effect as well as fatigue during the test. Consequently, in the follow up the output of the first recorded test is typically excluded and the development of a new VF defect requires confirmation with repeated testing. Also, the VF reliability indices, including: fixation losses, false negative and false positive errors should be taken into account when interpreting the results.

There is no widely agreed single reference standard for POAG diagnosis. Assessment of all these components is helpful in the diagnostic process but in some patients it can still be difficult to confidently establish the diagnosis of POAG, particularly in the early stages. Longitudinal monitoring with repeated structural and functional evaluation is often required to differentiate between glaucomatous and non-glaucomatous neuropathies, or indeed normal anatomical variants. In clinical practice a cohort of patients are described as 'glaucoma suspects' until the evolution of suspicious changes or the lack of it provides further clarification.

### *1.2.3 POAG pathophysiology and risk factors.*

The biological basis of glaucomatous neuropathy is complex and not fully elucidated. Several potentially relevant pathophysiological processes and risk factors have been identified. Among them, IOP plays a dominant role in the optic nerve injury. Currently, it is also the only treatable risk factor for POAG.

#### *1.2.3.1 IOP*

IOP is a dynamic parameter which shows circadian variability and changes with posture, fluid status and physical exercise (10). Independently of these factors IOP also varies considerably from person to person and has a wide range of 'population normal' values

of between 10 to 21 mmHg. POAG in the context of IOP elevated above this population norm is often described as high tension glaucoma (HTG).

The balance between aqueous humour production by the ciliary body and aqueous humour outflow by the trabecular meshwork and the uveoscleral outflow pathway is what determines IOP (6). Unlike in angle-closure glaucoma, where the approach to the trabecular meshwork is occluded by iridocorneal contact, in POAG there is no iridocorneal contact, however, there is increased resistance to the fluid flow in the trabecular meshwork (11). According to the 'mechanical' theory elevated intraocular pressure leads to axonal injury and the consequent death of RGCs. It has been proposed that this occurs through a biomechanical stress exerted on the optic nerve head which impedes the retrograde axoplasmic transport of essential neurotrophic factors and can trigger a signalling cascade which ultimately leads to apoptosis or autophagy of the neurone cells (6). The level of IOP is undoubtedly related to RGCs death. Numerous population-based studies have shown consistently that the prevalence of POAG rises exponentially as the level of IOP increases (12). Studies have also unequivocally demonstrated that reduction of IOP prevents the development of POAG in people with ocular hypertension and reduces the risk of progression in patients with already established glaucoma, including in those who have baseline IOP within the normal range (normal tension glaucoma-NTG) (13–17). Thus, IOP lowering is the mainstay of POAG treatment. It can be achieved by several methods, including: pharmacological therapy, laser and surgical interventions.

Pharmacotherapy is typically the first line treatment and is usually administered in the form of eye drops applied to the ocular surface. They work by improving the drainage or reducing the production of the aqueous fluid which results in lowering of the pressure in the eye. There are several different classes of hypotensive agents available with different mechanisms of action, administration regimens and varying side-effect profiles. Prostaglandin analogues primarily enhance uveoscleral outflow and, due to minimal systemic side effects, once daily application and effective reduction of IOP during the day and at night, are the most commonly used topical drugs. An alternative to prostaglandins are β blockers, which work by decreasing the production of aqueous humour by the ciliary body. These agents are also widely used but can cause systemic respiratory and cardiovascular effects and are less efficacious in IOP lowering at night and in people who take systemic β blockers. Among other categories of topical medications are: carbonic anhydrase inhibitors which reduce aqueous humour secretion, and α adrenergic agonists which work by both decreasing aqueous humour production and by increasing its drainage. A combination of these medications can be used but the

main limitation of pharmacotherapy is low long-term adherence to daily administration of eye drops for what is often asymptomatic disease.

Laser procedure is another treatment option for patients who require more aggressive IOP lowering, are not adherent to the eye drops or cannot tolerate them. There is also evidence in support of using laser trabeculoplasty as a first line treatment (18). Currently, the most commonly performed laser procedure is selective laser trabeculoplasty (SLT) which employs laser energy applied to the trabecular meshwork in order to induce remodelling of the extracellular matrix and increase aqueous outflow. In the majority of patients SLT is initially successful in lowering IOP but with time it may lose its effectiveness so that some patients require repeated procedures or other forms of treatment.

The most invasive method of IOP lowering is incisional glaucoma surgery which is typically performed under local anaesthetics although general anaesthesia may be required for some patients. Several different types of surgical procedures with varying degree of invasiveness and long-term efficacy have been developed. The general principle is to create an additional outflow pathway for the aqueous fluid which enhances the drainage. In trabeculectomy which has been the most frequently performed filtration surgery for over 40 years, this is achieved by the creation of fistula connecting the anterior chamber and the subconjunctival space (6). The aqueous fluid is then drained to a 'bleb' formed by a scleral flap and covered by the conjunctiva.

Surgical interventions result in reduction of IOP which is often sustained for many years without the need for taking eye drops but are expensive and not free from complications. For some patients, multimodality treatment is required to bring their IOP under control but even if this is achieved the glaucomatous damage may continue progressing. For example, in one landmark randomised interventional study in glaucoma which has shown the benefit of IOP lowering (the Early Manifest Glaucoma Trial), 45% of patients in the treatment arm (vs 62% of patients in the no treatment arm) still progressed during the median follow up of 6 years despite receiving a combined treatment with laser trabeculoplasty and a topical β blocker (14). It is also well recognised that in 30-50% POAG patients the IOP is within normal population range at least at the point of diagnosis (NTG) and only 10% of people with ocular hypertension will eventually develop glaucoma (19).

It is therefore important to identify other risk factors. So far, studies have shown that, apart from IOP, the main risk factors for glaucoma development and/or progression are: age, ethnic background, myopia and family history of glaucoma. Pooled data from population-based studies indicate that the risk of developing POAG increases by 1.73 (95% CI: 1.63 to 1.82) for every decade increase in age beyond 40 years (20). People

of African ancestry are 2.8 times more likely (95% CI, 1.83-4.06) to have POAG than people of European ethnic background (20). In addition, African-Americans tend to present with more severe visual field defect at younger age in comparison to white Americans (21). First-degree relatives of patients with POAG are several times more likely to develop the disease (22). A moderate to high degree of myopia is a risk factor for glaucomatous damage (23). Also, structural and biomechanical properties of the cornea (lower CCT=central corneal thickness and lower hysteresis) have been associated with faster POAG progression in some studies, although the evidence for these factors is weaker (23,24).

Identification and assessment of all these components in clinical practice allows for more accurate risk stratification so that aggressive IOP lowering can be implemented for patients at higher risk of progression. However, at least currently, none of these factors is modifiable. In contrast, impaired blood flow and oxidative stress which may also contribute to RGCs death are potential targets for disease modifying interventions.

*1.2.3.2 Vascular factors and oxidative stress*

It has long been proposed that vascular dysfunction promotes the optic nerve damage by either increasing the individual susceptibility to IOP or as an independent mechanism. According to the 'vascular' theory unstable perfusion pressure and impaired autoregulation result in insufficient blood flow in the ocular microvasculature and the consequent ischaemic injury as the metabolic demands of the neurone cells cannot be met (25). Retinal cells have a high metabolic rate and are particularly sensitive to disturbances in the supply of their energy sources, that is: oxygen, glucose and other metabolic substrates. Due to the requirement for relative optical transparency, the density of the vascular network which serves to meet this demand is constrained. Therefore, the blood flow must be precisely regulated as even minor disruptions in the homeostatic mechanisms can have deleterious consequences for the supplied tissues (26).

The anatomy and physiology of blood supply to the ONH are complex. The primary source of blood supply is the posterior ciliary artery circulation via the peripapillary choroid and short posterior ciliary arteries but the superficial layers of the ONH, including the RNFL, are supplied by small branches of the central retinal artery (27). Retinal vessels fully supply the central RGCs but the peripheral RGCs rely on both the retinal and choroidal vascular supply (28). These two vascular beds have different physiological properties (25,29). The retinal circulation has no autonomic innervation but otherwise resembles the brain circulation; tight endothelial junctions create a blood-retinal barrier and the blood flow is autoregulated within a certain range of perfusion pressure. The

autoregulation is at least partly based on partial pressure of oxygen and carbon dioxide. Blood flow level in the retinal circulation is low but high oxygen extraction ensures adequate tissue oxygenation. In contrast, choroidal microcirculation is characterised by high blood flow but low oxygen extraction. The endothelium of choroidocapillaries is fenestrated which means that relatively large vasoactive molecules such as endothelin-1 and angiotensin II have direct access to the smooth-muscle cells and pericytes of larger vessels and can also diffuse to the ONH. Further, choroidal circulation has poor autoregulatory properties and therefore is more dependent on perfusion pressure. It is also regulated by the autonomic nervous system.

It has been stipulated that this unique situation where some RGCs and their axons are dependent on both the retinal and choroidal capillaries for critical nutrition and oxygen, can render them more vulnerable to various insults affecting the vasculature particularly when, with increasing age, the autoregulatory and autonomic mechanisms become less robust (28).

There is now compelling evidence that vascular dysregulation exists in POAG and may be pathogenic. Whilst precise measurements of ocular blood flow, particularly in the microcirculation relevant to the optic nerve is challenging because of the complex angio-architecture, studies found deficiencies in retinal, choroidal and retrobulbar circulations in patients with glaucoma compared with individuals without the disease (30–32). Abnormal autoregulation of ONH and retinal blood flow in response to isometric exercise and posture change has been demonstrated in people with POAG and is more common than in healthy controls (33–35).

Moreover, there is evidence that the blood flow alterations are not limited to ocular circulation but represent more diffuse systemic vascular dysregulation present in POAG. For instance, studies which assessed flow mediated vasodilatation in the brachial artery, a marker of systemic endothelial dysfunction, have found that both NTG and HTG patients have reduced responses compared to controls which indicates impaired nitric oxide signalling (36–38). Endothelin-1, a potent vasoconstrictor, may also play an important role in vascular dysregulation in POAG. Intravitreal injection of endothelin-1 in animal studies induced chronic hypoperfusion in the ONH and led to thinning of the ganglion cell layer and loss of axons in the optic nerve (39–41). In a meta-analysis of 10 human studies, patients with NTG (but not those with HTG) had significantly higher plasma endothelin-1 levels compared to non-glaucomatous controls (42). People with HTG had however higher endothelin-1 levels in the aqueous humour. The authors concluded that in NTG there may be both a local and a systemic component in endothelin-1 mediated vascular dysregulation, while in HTG the effect of this peptide could be localised to the ocular vasculature. Another study has found that patients with

progressive POAG had higher serum endothelin-1 levels then those with stable disease (43). Endothelin-1 is constitutively expressed in different ocular structures and its role probably extends beyond the regulation of vascular tone. In vitro studies demonstrated that human trabecular meshwork cells contract in response to endothelin-1 which would increase aqueous outflow resistance and thus raise the IOP (44). Importantly, the synthesis and release of endothelin-1 are upregulated under hypoxic conditions and this process seems to be mediated by tumour necrosis factor α (TNF α) (45).

Also, there is evidence in support of generalised autonomic dysfunction in people with POAG. Several case-control studies of heart rate variability have shown that the autonomic balance is shifted towards higher sympathetic activity in POAG patients versus controls and that autonomic control of cardiovascular responses is blunted in NTG patients (46–50). Apart from vascular regulation the autonomic nervous system may also, at least partly, influence IOP fluctuation. In animal models, chemical stimulation of central autonomic regulatory neurons in hypothalamus increased IOP by nearly 70% from baseline (51).

More recently oxidative stress has been recognised as one of the main molecular mechanisms in the signalling pathway leading to RGCs death (6). The segments of RGCs axons which pass through the lamina cribrosa are not insulated by myelin sheaths which means that they require more energy to generate action potentials. This is reflected by high concentration of mitochondria in RGCs soma and the intra-retinal portions of their axons (52). Mitochondria are therefore critical to survival and function of RGCs but can be easily damage by oxidative stress and excessive formation of free radicals under hypoxic conditions and this process leads to apoptosis (53). Trabecular meshwork cells are also prone to degeneration by free radicles accumulating in the anterior chamber which causes cytoskeletal rearrangements and the consequent impairment in aqueous drainage (54). There are several potential sources of hypoxic stress in POAG, including mechanical stress imposed on the axons by high IOP, periods of relative ischaemia and reperfusion caused by vascular dysregulation and potentially systemic hypoxaemia. In support of the latter are data from a meta-analysis of 22 case-control studies which have shown significantly increased levels of oxidative stress markers not only in the aqueous humour but also in serum in POAG patients (55). In addition, depleted antioxidant potential has been demonstrated in serum of POAG patients and malonyldialdehyde was proposed as the best serum biomarker of oxidative stress in POAG. Importantly, this meta-analysis found that the differences in the serum level of oxidative markers are significantly higher between POAG and control patients than between angle-closure glaucoma and controls which would indicate that systemic

oxidative stress plays a more important role in the pathophysiology of POAG than in other types of glaucoma.

Various strategies, including already established and novel pharmacological agents, have been trialled to improve vascular dysregulation and reduce oxidative stress as ways of complementing IOP lowering treatment but so far none of them have made it to clinical practice (42,56,57). Attenuation of the underlying risk factors which promote the oligaemic/hypoxic injury in the ONH would be an ideal way of preserving the RGCs. A common and treatable disorder which, among other potentially relevant pathophysiological consequences, causes vascular dysfunction and induces systemic oxidative stress is obstructive sleep apnoea (OSA).

### *1.3 OSA*

#### *1.3.1 What is OSA?*

OSA is a common sleep-related breathing disorder (SBD) characterised by recurrent episodes of complete (apnoea) or partial (hypopnoea) occlusion of the upper airway. Occasional nocturnal obstructive respiratory events are normal but when their frequency increases to ≥5 per hour of sleep (the current diagnostic threshold for OSA) they can be associated with daytime symptoms and adverse health outcomes (58). Obstructive apnoeas and hypopnoeas cause transient interruption of ventilation and are usually terminated by brief arousals (microarousals) which disrupt sleep continuity. Interrupted ventilation is associated with oxygen desaturations, intrathoracic pressure swings and sympathetic drive activation. Collectively, these physiological changes and sleep fragmentation lead, in turn, in some individuals, to a range of symptoms including: excessive sleepiness, impairment of memory and cognition, mood alterations, and changes in driving competence (59–64). However, a substantial proportion of people, even those with severe OSA are not symptomatic or aware of having a sleep disorder (65,66). On the contrary, about 20% of people report excessive daytime sleepiness in the absence of SDB (58). For this reason OSA cannot be diagnosed based on symptoms or subjective reports alone. A sleep recording, either a full polysomnography (PSG), which consist of: electroencephalography, electrooculography and electromyography in addition to respiratory and cardiac signals, or a respiratory polygraphy (rPG) which does not include the neurophysiological monitoring is required to confirm or refute the diagnosis of OSA. In some patients nocturnal oximetry alone can be sufficient to diagnose OSA but it is less sensitive as it allows detection of only these respiratory events which are associated with 4% oxygen desaturations and cannot reliably differentiate between different types of SDB.

OSA is one of the most common chronic disorders and a major public health issue in most countries (67). In a recent systematic review, the overall population prevalence of OSA ranged across the studies from 9% to 38% but was higher in men and elderly (68). Moreover, its prevalence increases worldwide in line with rising rates of obesity. For instance, based on longitudinal data from the Wisconsin cohort, it has been estimated that over the last two decades the prevalence of moderate to severe OSA has increased by 14-55%, depending on sex and age subgroups (69).

Apart from causing sleepiness and affecting quality of life in some individuals, OSA is associated with cardiovascular, cerebrovascular and metabolic co-morbidities as well as increased risk of certain cancers and all-cause mortality (70–75). In fact, OSA is now considered as a systemic disorder which can affect most, if not all, organs (76).

Obesity is the strongest risk factor for OSA. Even modest changes in weight can worsen OSA severity. For instance, in a longitudinal study by Peppard et al. 10% weight gain has been associated with a 32% increase in apnoea-hypopnoea index (AHI) (95% CI 20-45%) and a six folds increase in the odds of developing moderate to severe OSA (AHI>15 events/hr) but 10 % weight loss has led to a 26% (95% CI 18-34%) reduction in AHI (77). Several mechanisms have been proposed to explain the contribution of excessive weight to the development of OSA including deposition of fat around pharyngeal airways and particularly in the tongue, reduction of functional residual capacity as well as low lung volumes leading to decreased oxygen reservoir and ventilatory control instability (78,79). Nonetheless, approximately a third of OSA patients are not obese and not all obese patients, even those with large neck circumferences suffer from OSA (80). From the pathophysiological perspective, OSA is a multifactorial disease although the combination of identified anatomical and non-anatomical factors and their relative contributions vary between individuals. In some, excessive weight, craniofacial or soft tissue changed play major roles, in others non-anatomical factors, such as impaired pharyngeal dilator muscle function, low respiratory arousal threshold, and high loop gain causing unstable control of breathing may be the predominant mechanisms leading to upper airway instability during sleep (81).

In general, OSA is twice more common in men than in premenopausal aged-matched women (82). The reasons for male predominance are not fully elucidated but hormonal influences on breathing control and upper airway muscle activation, relatively longer pharyngeal airway and sex-specific fat distribution may explain some of this disparity. Hormonal changes and redistribution of fat tissue to the upper body, including the neck, could be responsible for a relative increase in OSA after the menopause in women (83,84).

Age is also important in OSA epidemiology and pathophysiology. The prevalence of OSA increases with age up until the age of 65 years when it seems to plateau (85,86). Preferential deposition of fat around the pharynx, independent of systemic fat and deteriorating genioglossus negative pressure reflex have been proposed as potential mechanisms of increased upper airway collapsibility with aging (87,88). Most patients are however diagnosed with OSA between the age of 40 and 50 years and the association of OSA with sleepiness and vascular co-morbidities such as hypertension seems weaker in elderly people (89–91). Thus, it has been suggested that OSA may represent a different entity in older age groups but, as most studies to date predominantly included young to middle-aged adults more data are needed to better understand the differential effect of age on OSA consequences (92).

### *1.3.2 Pathophysiological mediators of OSA*

Recurrent obstructions of the upper airway activate a range of stressors which contribute to cardiac, vascular and metabolic diseases. Transient hypoxaemia and hypercapnia are inherently associated with obstructed breathing. The hypoxaemic stress is further augmented by the subsequent re-oxygenation which results in the generation of reactive oxygen species (ROS). Excessive production of ROS cannot be counterbalanced by the limited capacity of the anti-oxidant systems and results in hypoxic stress, which is thought to promote inflammation and sympathetic activation. These, in turn, potentiate the oxidative stress creating a vicious cycle which may ultimately lead to endothelial dysfunction, hypertension and atherosclerosis (93). Complex cellular and molecular mechanisms are involved in increased ROS production in response to hypoxia, including: mitochondrial dysfunction, activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and xanthine oxidase, and uncoupling of nitric oxide synthase, which generates ROS rather than nitric oxide (94).

Biomarker studies in OSA patients have provided evidence for enhanced release of superoxide from leucocytes, diminished bioavailability of nitric oxide, reduced antioxidant capacity and increased peroxidation of lipids and oxidative DNA damage (95). Notably, marked mitochondrial dysfunction in animal models of intermittent hypoxia has been associated with apoptotic cell death in the cerebral cortex (96). An abnormal mitochondrial distribution and impaired oxidative activity have also been demonstrated in the soft palate muscles of OSA patients (97).

Various blood cells of people with OSA exhibit a pro-inflammatory and a pro-thrombotic phenotype with a higher expression of adhesion molecules and inflammatory cytokines, such as interleukin-8 or TNFα, which promote endothelial injury and thrombosis (94). Endothelial dysfunction is well documented in OSA at least at the macrovascular level

(98). The bioavailability of nitric oxide, a key vasodilator, is reduced and accompanied by increased production of endothelin-1, a potent vasoconstrictor (99,100).

Intermittent hypoxia and hypercapnia alter chemo- and baro-reflexes and this leads to augmented sympathetic activity in sleep which carries over to wakefulness contributing to nocturnal and diurnal hypertension and blunted heart rate variability (101).

The mechanical consequence of obstructed breathing events are acute intrathoracic pressure swings which stretch intrathoracic structures, in particular, the atria. They induce haemodynamic and electrophysiological alterations and may promote arrhythmias (102).

All these mechanisms and cellular pathways are interconnected and thought to be collectively responsible for OSA related co-morbidities.

### *1.3.3 OSA treatment*

OSA treatment is directed to alleviating symptoms, improving quality of life, reducing the risk of road traffic collisions and, at least in some patients, reducing the burden of cardiovascular co-morbidities.

General therapeutic options include: lifestyle modifications, such as reduction in body weight, abstinence from alcohol and positional interventions to prevent sleeping in a supine position (103,104). Oral appliances can be used to control mild to moderate OSA, if feasible from a dental perspective (105). For highly selected individuals maxillofacial surgery is a valid treatment option but it is rarely used due to its invasive nature (106).

For the majority of people however, the usual first line treatment, particularly in the context of moderate to severe OSA, is continuous positive airway pressure (CPAP) which is applied via a nasal or a full face mask to splint open and maintain patency of the upper airway throughout the breathing cycle. When used throughout the entirety of sleep, CPAP can eliminate nearly all obstructive breathing events in the majority of patients (107,108). CPAP therapy has been shown to have positive impacts on several of the deleterious pathophysiological effects of OSA, including; ameliorating sympathetic overactivity, improving endothelial function, eliminating negative intrathoracic pressure swings and, in some studies, reducing circulating markers of systemic inflammation (109–112). The effect of CPAP on oxidative stress is less clear; the majority of observational studies have shown a significant decrease in the biomarkers of oxidative stress and an increase in antioxidant capacity but randomised sham controlled trials have yielded contradictory results (113–115). There is little doubt that consistent use of CPAP improves the quality of sleep, normalises sleep architecture, reduces daytime sleepiness, enhances neurobehavioural performance and eliminates the increased risk of motor vehicle crashes (61,116,117). Patients who are compliant with CPAP achieve

better blood pressure control and this effect is larger in those with resistant hypertension (118,119). Whether CPAP reduces the risk of cardiovascular events and mortality is still uncertain. Multiple observational studies have indicated the cardiovascular benefits of CPAP but this has not been confirmed in randomised controlled trials (RCTs), possibly due to suboptimal CPAP usage (120).

### *1.4 POAG and OSA Associations*

#### *1.4.1 Hypothetical pathophysiological links*

Through intermittent hypoxia and hypoxic stress promoting RGCs apoptosis, endothelial dysfunction and sympathetic hyperactivity affecting blood flow regulation in the ocular vessels, intrathoracic pressure swings potentially causing IOP fluctuations and transient hypercapnia altering intracranial pressure, OSA may directly or indirectly compromise the optic nerve head perfusion and oxygenation contributing to RGCs degeneration (121,122).

It is remarkable that many of the physiological deficits which characterise OSA have also been found in people with POAG. These observations are, of course, not sufficient to conclude that OSA is behind these changes in POAG patients or indeed that it contributes to glaucomatous neuropathy, but the overlapping nature of these findings and theoretically plausible physiological mechanisms prompt further exploration of the potential associations between OSA and the optic nerve damage.

#### *1.4.2 Associations of OSA with ocular parameters*

Previous studies have largely examined cross-sectional associations between OSA metrics and parameters of the optic nerve structure and function in people with otherwise healthy eyes. Most of these studies have shown a thinner RNFL, reduced retinal sensitivity (lower mean deviation-MD) and impaired electrophysiological properties (delayed latency and reduced amplitude of the P100 waveform on visual evoked potential testing) when compared with people without OSA, although, in some studies these observations have been limited to severe OSA only and data on RNFL thickness are not unanimous (123–125). As already alluded to, testing retinal sensitivity with perimetry is problematic in people with OSA as their performance may be affected by impaired attention and decreased reaction times, giving falsely underestimated visual field indices. Measurements of RNFL thickness with OCT devices allows for a more objective assessment with less variability and so would be preferred over functional testing to assess for the impact of OSA.

The above associations have not to date been examined in people with already established POAG. Such studies are more challenging as they require a relatively large sample of participants with well characterised glaucoma to enable adjustment for IOP and other glaucoma risk factors.

Critically, there are no longitudinal data on RNFL thickness change over time in people with and without OSA, and therefore it is unclear whether OSA contributes to POAG progression.

### *1.4.3 Prevalence of OSA in POAG*

Most of the studies to date have focused on examining the prevalence of glaucoma in people with OSA. Three meta-analyses of these studies have concluded that OSA is a risk factor for the development of glaucoma but, as many of the included studies did not adjusted for known risk factors or OSA severity, were retrospective and at high risk of selection bias, the authors rightly attributed low confidence to these data (126–128). The additional problem faced even by prospective studies is that, for the reasons outlined in paragraph 1.2.2, it is often difficult to confidently diagnose POAG based on a single examination and the previous cross-sectional studies have not offered a repeated ophthalmic evaluation. There are only two longitudinal controlled studies, both showing increased risk of subsequent POAG diagnosis in people with OSA, but they were based on retrospective review of data derived from health insurance databases (129,130).

Several controlled studies have assessed the prevalence of OSA in people with glaucoma. OSA was reported in up to 42% of patients with POAG, which was thought to be higher than in the general population (131). These studies were also summarised in a recent meta-analysis (128). Based on a fixed-effects model, the pooled OR of OSA was 1.96 (95% CI 1.37- 2.80) among POAG patients vs controls. However, the individual studies included had important methodological limitations related to small sample sizes, pre-selecting patients based on symptoms, using only low grade sleep tests or questionnaires alone, or ICD (International Classification of Diseases) codes in retrospective health record linkage designs. One particular problem with database retrospective studies is that both POAG and OSA are largely underdiagnosed in the general population and hence identification of these patients based on ICD codes (diagnosed cases only) has limited value even in relatively large samples. These studies are summarised in Table 1-1 which also describes one further study published more recently which used the STOP-BANG questionnaire to assess for the risk of OSA (132). Because of all these limitations, it is still unclear whether OSA is truly more prevalent in people with POAG. In addition to prevalence, it is important to establish the burden of

OSA symptoms and the distribution of OSA severity across different stages of visual impairment in POAG. There may be a dose response effect in the possible impact of OSA on glaucoma; mild OSA may not be at all relevant. Furthermore, OSA may be contributing to the pathogenesis of glaucoma only in certain POAG subgroups, such as NTG where the glaucomatous damage seems less IOP dependent. On the contrary, exposure to intermittent hypoxia and impaired blood flow may have a synergistic effect in HTG where raised IOP is already posing mechanical stress on the optic nerve and its vasculature.

OSA is a treatable condition and if proven to be an independent risk factor for glaucoma, those patients diagnosed with glaucoma may benefit from routine screening and potentially from treatment for OSA. However, screening of unselected patients in busy glaucoma clinics with complex sleep tests is unlikely to be feasible or cost-effective. Therefore, there is a need to evaluate tools such as questionnaires, for their usefulness in identifying patients at a higher risk of OSA.

Table 1-1. Summary of studies that assessed the prevalence of OSA in people with POAG.

| Study | Participants | Diagnostic test | % with SDB diagnosis |
|---|---|---|---|
| Onen 2000(133) | 212 POAG<br>218 Controls | Semi-structured questionnaire | POAG-48% snoring, 27% snoring + EDS<br>Controls- 38%snoring, 17.8% snoring + EDS |
| Marcus 2001(134) | 23 NTG<br>14 NTG suspects<br>30 Controls | 'sleep history',<br>PSG only on selected subjects | NTG-30%<br>NTG suspects-14.3%<br>Controls-3% |
| Girkin 2006(135) | 667 Newly diagnosed Glaucoma<br>6667 Controls | Unknown,<br>diagnosis based on ICD code | Glaucoma-1%<br>Controls-0.5 |
| Khandgave 2013(136) | 40 POAG<br>40 Controls | Sleep disturbance questionnaire + ESS,<br>PSG only on selected subjects | Glaucoma-10%<br>Controls-2.5% |
| Roberts 2009(137) | 52 Glaucoma<br>60 Controls | Oximetry | Glaucoma-17%<br>Controls-12% |
| Bilgin 2014(131) | 24 NTG<br>24 Controls (matched) | PSG | NTG-41.7%<br>Controls-12.5% |
| Cabrera 2018(132) | 437 POAG<br>441 Controls | STOP-BANG questionnaire | POAG-62.7% at high risk of OSA<br>Controls-59.4% at high risk of OSA |

Abbreviations: POAG=primary open-angle glaucoma, NTG=normal tension glaucoma, PSG=polysomnography, OSA=obstructive sleep apnoea, SDB=sleep-disordered breathing, ESS=Epworth Sleepiness Scale Score, EDS=excessive daytime sleepiness.

### *1.4.4 CPAP, IOP and ocular microvasculature*

Many people with glaucoma and concomitant OSA associated with the relevant symptoms, receive standard treatment with CPAP. However, the impact of CPAP on their glaucoma is unknown. On one hand CPAP, by eliminating the negative consequences of OSA, may have a protective effect on RGCs and their axons. On the other hand, there are concerns that it raises IOP. The evidence for this is limited and comes from two studies which only examined people without glaucoma (138,139). I will describe these studies in detail in the relevant chapters but, briefly, they have found that IOP increases significantly during the nocturnal CPAP therapy compared to the pre-treatment baseline. People with glaucoma, particularly those with HTG, may be more susceptible to IOP change when on CPAP and in comparison to normal subjects may experience a greater increase in IOP. Furthermore, the relationship between CPAP level and IOP has not been studied. It is unknown if IOP increases in a correlated way to CPAP or whether there is no straightforward correlation. If the first is true, application of CPAP only up to a certain pressure level would be safe and perhaps the threshold to use other treatment modalities, such as bi-level positive airway pressure which is equally effective but allows for application of lower pressure in the expiratory phase of the breathing cycle, should be lower in patients with glaucoma. If, however, IOP changes are a matter of individual response to CPAP, perhaps dependent on body mass index (BMI), lung volumes or OSA severity, a routine measurement of IOP should be performed once CPAP is started. This is currently not a part of standard clinical practice. It is also possible that CPAP set within the pressure range used in clinical practice does not influence IOP or its effect is not mediated by simple mechanical pressure transmission from the airways to the ocular veins, as it has been proposed (138).

Understanding the relationship between CPAP and IOP is important as it may inform the management of people with OSA and concomitant glaucoma. In fact, this research is in part inspired by a patient with glaucoma who was diagnosed with OSA and attended one of our sleep disorders clinics to start CPAP therapy. One of her questions was whether CPAP would affect her IOP and this was a question we could not answer.

Apart from IOP, CPAP can potentially influence ocular blood flow and the microcirculation of the optic disc. Again, it may have a negative effect by increasing IOP and thus reducing ocular perfusion pressure or positively influence the blood supply by improving endothelial function and autoregulation of the ocular blood flow. New, non-invasive imaging techniques, such as OCT angiography, allow for a direct and precise assessment of ocular microvasculature and could provide useful data on the vascular effects of CPAP in glaucoma and beyond.

## *1.5 Objectives of the Thesis*

*Chapter 3*

The work described in Chapter 3 is dedicated to establishing what proportion of unselected patients with POAG attending glaucoma clinics in a secondary care hospital meet the diagnostic criteria for OSA. Their symptoms and OSA severity will be examined and compared with a concurrent group of individuals without POAG after matching for known OSA risk factors. Further, I will examine for associations between OSA severity markers and indicators of functional and structural optic nerve damage while controlling for other glaucoma risk factors. The burden of OSA in terms of its prevalence and severity will be compared between patients with NTG and HTG. I will also assess the usefulness of the STOP-BANG questionnaire as a screening tool for OSA in POAG patients.

*Chapter 4*

Based on longitudinal data extracted from electronic health records of patients who participated in the study reported in Chapter 3, this Chapter will examine whether the rates of structural POAG progression (RNFL thinning) are different between patients with glaucoma, with and without OSA, and whether OSA could be an independent risk factor for POAG progression.

*Chapter 5*

The work reported in Chapter 5 seeks to establish whether CPAP therapy increases nocturnal IOP in patients with POAG. The potential mediators of IOP response to CPAP will be examined and the effect of CPAP of IOP will be compared between POAG patients and control subjects.

*Chapter 6*

This Chapter will examine the effect of the acute application of CPAP on IOP in people with treated and untreated POAG as well as in control subjects. The specific aim of this Chapter is to establish whether there is a relationship between applied CPAP level and the level of IOP.

Chapter 7

The work described in Chapter 7 will explore whether a short course of CPAP therapy in people with OSA with and without POAG can alter ocular microvascular parameters measured with a novel angiographic device.

Chapter 8

This Chapter will summarise the main results of all previous chapters and will discuss directions for future work.

# Chapter 2: Materials and Methods

This Chapter describes general methods used across the studies, including; research settings and population, data collection and management, outcome measurement tools, funding sources as well as my own and others' contributions to each study. Specific procedures are detailed separately in the relevant chapters.

*2.1 Research settings and population*

For the purpose of this thesis an interdisciplinary collaboration between sleep specialists and ophthalmologists was established. The studies were conducted across two sites: the Respiratory Support and Sleep Centre (RSSC) at the Royal Papworth Hospital NHS Foundation Trust and the Huntingdon Glaucoma Diagnostic & Research Centre based in the Department of Ophthalmology at Hinchingbrooke Hospital-North West Anglia Foundation Trust.

RSSC is an accredited, tertiary sleep disorders unit with a national referral base and specialist expertise in the diagnosis and management of OSA and other sleep disorders. The Huntingdon Glaucoma Diagnostic & Research Centre was established in 2007 with a research team consisting of consultant ophthalmologists supported by research optometrists, orthoptists and research nurses. The unit conducts the majority of glaucoma clinical studies (both commercial and non-commercial) in Eastern England.

Participants who took part in the POSAG study (Chapter 3) were recruited among patients who attended glaucoma clinics at Hinchingbrooke Hospital and their relatives. All study procedures took place in a specially set up Glaucoma Research Clinic held on three days a week for 18 months. Sleep studies were analysed in the RSSC.

Recruitment to research studies described in Chapters 5,6 and 7 took place predominantly in the RSSC, with a prior screening and ocular examination for some participants carried out at Hinchingbrooke Hospital as required.

*2.2 Data collection and management*

With the exception of the retrospective study described in Chapter 4, data for all other studies were collected prospectively and recorded in electronic Case Report Forms (eCRF) developed in OpenClinica which is an electronic data repository currently preferred by the Sponsor (Royal Papworth Hospital) for data capture and management. For each study, eCRFs were designed based on the study protocol and built with incorporated validity checks to minimise data entry errors. Data integrity was monitored throughout the study by the data management team from the Sponsor's institution. In

addition, the accuracy and consistency of data entry were audited by the Sponsor's representative. Adverse and Serious Adverse Events were reported electronically using the eCRFs and in accordance with the Trust Standard Operating Procedures and the principles of Good Clinical Practice.

*2.3 Outcome measurement tools*

Across the studies the following tools were used to examine the main outcomes:

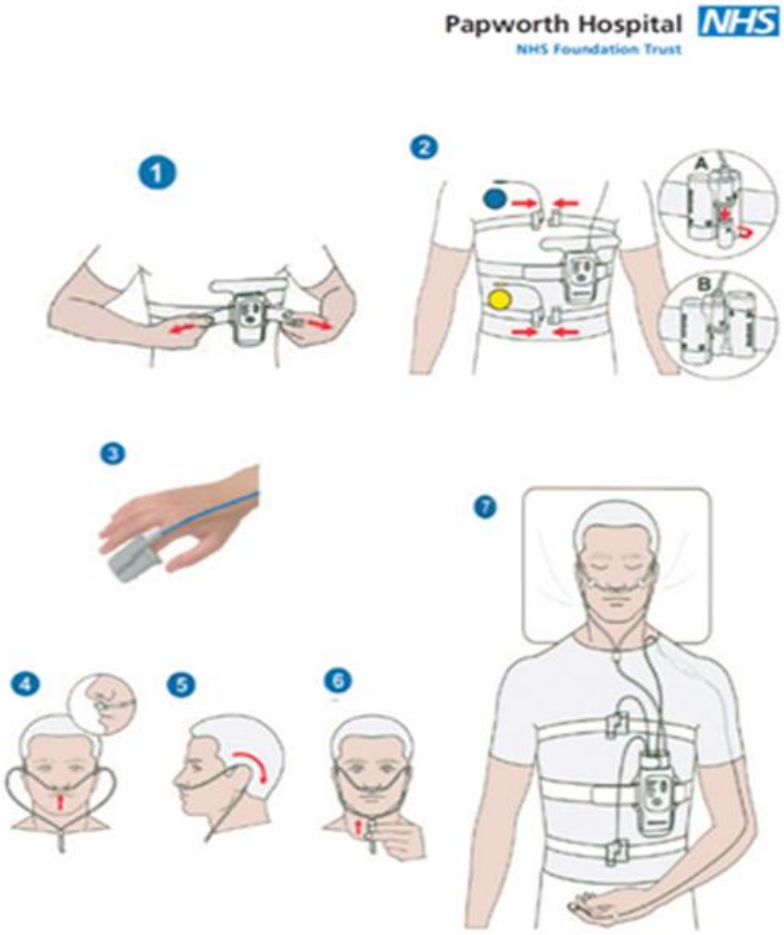
- **Respiratory polygraphy (rPG)** was the chosen sleep test for OSA screening. It was performed using the Embletta portable device (Embletta MPR and GOLD; Natus Medical Inc., Pleasanton, USA) with the following channels (Figure 2-1):

i. Airflow measured by thermistor and nasal pressure-flow cannula
ii. Thoracic and abdominal effort through XactTrace Respiratory Inductive Plethysmograph sensors
iii. Continues pulse-oximetry
iv. Body position determined by a sensor which collects X/Y/Z gravity information.
v. Activity sensor which detects movements.

The sleep study kit was collected from the participants on the day after the sleep test and data were downloaded using RemLogic-E software to assess whether the study was of adequate quality. All sleep studies were then scored manually in the RSSC by a single qualified sleep polysomnographer and in accordance with current American Academy of Sleep Medicine guidelines(140). Briefly, apnoeas were scored when there was a drop in the peak signal excursion by ≥ 90% of the pre-event baseline for ≥10 seconds using an oro-nasal thermal sensor and hypopneas were defined as ≥ 30% reduction in the pre-event nasal pressure signal which lasted for ≥10 seconds in association with ≥ 3% oxygen desaturation. Apnoeas were classified as obstructive, mixed, or central based on the respiratory effort (see Appendix 1 for an example of a multichannel recording demonstrating obstructive apnoeas).

The diagnosis of OSA was given if the apnoea-hypopnoea index (AHI, the number of apnoea and hypopnoea events per hour of recording) was greater than or equal to 5 and the majority of respiratory events were obstructive in nature. The usual clinical thresholds were used to categorise OSA severity: mild (AHI≥5 to <15), moderate (AHI≥15 to <30), severe (AHI≥30).

Figure 2-1. Illustration of rPG (Embletta) montage.



- **The Epworth sleepiness scale** (ESS) was developed by Dr Murray Johns of Epworth Hospital in Melbourne, Australia and first introduced in 1991 (141). The ESS is now an internationally accepted scale used to measure the subject's general level of daytime sleepiness. It estimates the chance of falling asleep in eight different situations where subjects rate their tendency to fall asleep on a scale of 0 to 3; 0= no chance of dozing, 1=mild chance, 2=moderate chance and 3 a high chance of dozing (Figure 2-2). A score of <11 is considered to be normal and a value of ≥ 11 suggests excessive daytime sleepiness.

Figure 2-2. The Epworth Sleepiness Scale adopted by the RSSC.

**Epworth Sleepiness Scale**

How likely were you to fall asleep in the following situations, in contrast to just feeling tired?

This refers to your usual way of life in the last four weeks. Even if you have not done some of these things recently try to work out how they would have affected you. Use the following scale to choose the most appropriate number for each situation.

0 = Would never doze
1 = Slight chance of dozing
2 = Moderate chance of dozing
3 = High chance of dozing

| SITUATION | 0 | 1 | 2 | 3 |
|---|---|---|---|---|
| Sitting and reading | | | | |
| Watching TV | | | | |
| Sitting inactive in a public place (e.g. a theatre or a meeting) | | | | |
| As a passenger in a car for an hour without a break | | | | |
| Lying down in the afternoon (when circumstances allow) | | | | |
| Sitting and talking to someone | | | | |
| Sitting quietly after lunch without alcohol | | | | |
| In a car, while stopped for a few minutes in traffic | | | | |

- **The STOP-Bang questionnaire** was developed and validated as a pre-operative screening tool for assessing the risk of OSA in general surgical population in 2008 by Chung *et al* (142). Since then it has been validated in several different populations and settings, such as among bus drivers or in primary care (143). It is a concise and user-friendly tool comprising eight equally weighted questions with binary responses; each positive response scores one point. A high risk of sleep apnoea is defined as a score of ≥3 and a low risk as a score 0-2 (Figure 2-3).

Figure 2-3. The STOP-Bang Questionnaire.

Name: Date:

**STOP-BANG Questionnaire**

| | | |
|---|---|---|
| 1. *S*noring. Do you snore loudly (louder than talking or loud enough to be heard through closed doors)? | Yes ☐ | No ☐ |
| 2. *T*ired. Do you often feel tired, fatigued or sleepy during the daytime? | Yes ☐ | No ☐ |
| 3. *Observed*. Has anyone observed you stop breathing during your sleep? | Yes ☐ | No ☐ |
| 4. Blood *P*ressure. Do you have or are you treated for high blood pressure? | Yes ☐ | No ☐ |
| 5. *BMI*. BMI more than 35kg/m$^2$? | Yes ☐ | No ☐ |
| 6. *Age*. Age over 50 years old? | Yes ☐ | No ☐ |
| 7. *Neck* Circumference. Neck circumference greater than 40 cm? | Yes ☐ | No ☐ |
| 8. *Gender*. Male gender? | Yes ☐ | No ☐ |

High risk of OSA: Answering yes to three or more items

Low risk of OSA: Answering yes to less than three items

TOTAL ☐

- **Humphrey Field Analyser (HFA)** (Carl Zeiss Meditec, Jena, Germany) is the most commonly used perimeter for visual field assessment in glaucoma clinics in the UK. HFA quantifies the sensitivity of a patient's peripheral vision using standardised testing algorithms. All visual field examinations described in this Thesis were performed using the 24-2 tests pattern and the Swedish Interactive Thresholding Algorithm fast protocol (SITA Fast) which is the preferred method of VF assessment in the local glaucoma clinics. It is a fast threshold test which usually takes approximately 5 minutes per eye and offers high accuracy whilst reducing patient fatigue-related errors. The visual field is tested up to 24 degrees temporally and 30 degrees nasally from fixation. The threshold value for each of the tested location within the field represent the extent to which light can be dimmed and still detected by the patient and are referenced to age-matched normal population values (144). These multiple location-based data can then be aggregated and presented as global indices which provide a useful summary of the field. The Mean Deviation (MD) is derived from the Total Deviation and represents the overall mean departure of the test subject's visual field sensitivity from the age-corrected norm (145). Negative values indicate field loss whereas positive values indicate that the field is above average. The Pattern Standard Deviation (PSD) summarises localised loss while correcting for an overall depression in light sensitivity. For this reason it is thought to be particularly useful in patient with cataract where lens opacity can cause excessive light scatter. The Visual field index (VFI) is also less affected by media opacity and has been developed to better correlate with RGCs loss by giving more weight to the points near the centre of the field. The VFI value of 100% represents a perfect age-adjusted visual field and 0% equates to perimetric blindness. These global field indices have been extracted for the purpose of this Thesis (see Appendix 2).

- **Ocular Coherence Tomography (OCT)** is a non-invasive imaging technique which uses low-coherence interferometry to produce two- and three- dimensional cross-sectional images of optical scattering from internal tissues (146). As an echo technique it is analogue to ultrasound imaging but since it uses light rather than sound waves the produced images have much higher longitudinal and lateral spatial resolution which is down to the level of a few micrometres. Spectral domain (SD)-OCT is the latest iteration of the OCT technology. In comparison to other OCT modalities, it offers an increased axial resolution and a faster scanning speed that result in higher density scans and decreased motion artefacts (147). Various ocular structures and retinal layers can be accurately assessed with the SD-OCT but the most widely used parameter in glaucoma research and clinical practice for the disease detection and progression monitoring is circumpapillary RNFL thickness measurement. It enables a cross-sectional assessment

of all the RGCs' axons as they come together to form the ONH. For these reasons, the circumpapillary RNFL thickness has been selected in this Thesis as the primary parameter for assessment of structural glaucomatous damage and its progression.
The images were acquired with the OCT Spectralis (Heidelberg Engineering Inc. MA, USA.), an SD-OCT device which uses 1024 A-scan points from a 3.45 mm circle centred on the ONH. RNFL thickness is automatically calculated by proprietary software (version: 6.3.4) as the distance between the internal limiting membrane and the posterior border of the RNFL. The software output includes the average RNFL thickness for the whole globe and separately for each of the four quadrants (superior, inferior, nasal, and temporal) (148) (see Figure 4-1 in Chapter 4).

- **OCT Angiography (OCT-A)** is a novel functional extension of OCT which allows visualisation and quantitative assessment of retinal vasculature. This technique is based on high-speed OCT scanning and analysis of signal decorrelation between multiple sequential B-scans of the same cross-section of the retina (149). Blood vessels are defined by areas with high decorrelation and a static tissue has low decorrelation values (150). Unlike in conventional angiography there is no need for an extrinsic contrast as the signal is generated by the movement of erythrocytes within the vessels. Precise three-dimensional images of the vascular networks down to the level of capillaries are obtained and can be displayed as individual layers which allows for isolation of specific areas of interest (see Appendix 3). Importantly, these images are morphologically comparable to microscopic images of the histological specimens (151).
The OCT-A device (AngioVueHD, Optovue Inc., Fremont, CA) used in this Thesis for the angiographic measurements has been validated in multiple recent studies. It has the capacity for automated and quantitative evaluation of the optic disc, peripapillary and choroidal vessels density. The Angiovue software (version: 2017.1.0.151) calculates vessel density as the percentage of the measured area occupied by 'flowing' blood vessels which are defined by pixels with decorrelation values above a set threshold level. The measurements can be obtained with high within-visit, between-visit and interoperator reproducibilities (152–154).

- **Icare tonometers** (IcarePro and IcareHome, Finland, Oy) were used for repeated IOP measurements in studies described in Chapters 6 and 7. These are new generation handheld devices constructed based on the principles of rebound tonometry. Briefly, a small probe held in place by an electromagnetic field is accelerated against the cornea. It makes a brief contact with the corneal surface and then bounces back. The deceleration is faster if the IOP is high and slower if the IOP is low. The device measures

the rebound acceleration and converts it into IOP (155). For each IOP measurement six consecutive readings are obtained either by pressing the measurement button individually six times or by activating six rapid automatic measurements in a 'series' mode. The value of the final IOP is calculated as the average of four measurements after discarding the highest and the lowest readings. IcarePro displays the measurements on the device panel with one decimal place and, in addition, indicates the reliability of the measurements by a colour code; if the variation is within normal limits the colour is green, if it is greater than normal the indicator is yellow and red when the variation is unacceptably high. The IcareHOME which is designed for self-measurement of IOP does not display the final IOP but informs the patient by a beep signal and a light illuminated on the back panel whether the obtained measurements are acceptable or whether they need repeating. The final IOP readings are stored in the device internal memory and can be subsequently downloaded by a healthcare professional using a dedicated software (see Appendix 4)

Both devices have been validated against the Goldmann applanation tonometer which is still regarded as the gold standard for IOP measurements and have been shown to have good agreement (156–159). Rebound tonometry has important advantages over applanation tonometry. There is no need for application of topical anaesthetics and fluorescein which reduces the measurement acquisition time to a few seconds and minimises discomfort to the patient. The rebound technique does not require repeated calibration and is generally less operator dependent. Furthermore, an inclination sensor built in the IcarePro tonometer enables for the measurements to be performed also in a supine position. These characteristics made these rebound tonometers particularly suitable for the studies conducted within this Thesis.

*2.4 Contributions to the Thesis*

My personal and others' involvement in each study described in this Thesis was as follows:

Chapter 3:

- With help and supervision from Dr Ian Smith and Professor Rupert Bourne I have designed this study and developed the study protocol.
- With support of my co-applicants (Dr Ian Smith and Professor Rupert Bourne) I have successfully applied for an external grant (Fight for Sight, 15K) which secured the necessary additional funding for this project. Dr Ian Smith was previously awarded an unrestricted research grant from Breas Medical which covered most of the expenses of the research projects reported in this Thesis, including my salary.
- I have applied for ethical (NHS REC and ARU), HRA and Sponsor's (Royal Papworth Hospital) approvals and have also secured NIHR CRN Portfolio status.
- I have participated in the contract negotiations between the Trusts, prepared the study costings and drafted the contract with help from Dr Victoria Stoneman.
- I have designed eCRFs and prepared all study documentation. Jo Steel has programmed the study electronic data repository in OpenClinica and provided support with data managements throughout the study.
- I and Paula Turnbull, Dr Shabbir Harun, Aaron Woods and Professor Rupert Bourne have been involved in screening glaucoma patients and their relatives in glaucoma clinics.
- I have contacted nearly all potential participants and scheduled the study visits for those willing to take part. Paula Turnbull provided administrative support with visits organisation and maintenance of study documentation.
- I have participated in recruitment of all participants and performed the relevant sleep apnoea assessments with set up and evaluation of rPG studies, taken the relevant ophthalmic and medical history, performed anthropomorphic measurements and administered the questionnaires. The ophthalmic tests were performed by: Jane Kean, Dr Gil Peretz, Dr Catherine Willshire and Aaron Woods.
- I have extracted all data and recorded in the electronic repository.
- All sleep studies were scored by Sam Moir. In the process, I have also learnt scoring of rPG studies and have been formally signed off as competent in this task.

- I have reported the results of sleep studies to all participants and advised GPs accordingly.
- I have performed data analysis including all statistical analyses with the exception of propensity matching which was done by Dr Sofia Villar and Dr Yi-Da Chiu who also verified some of the regression models constructed by me. Dr Jules Hernandez-Sanches previously provided guidance on the study sample calculation.
- I have written this chapter and prepared the first draft of the manuscript. Dr Ian Smith and Professor Rupert Bourne provided critical review and edited the manuscript.
- I have reported to the study funders, the HRA and the Ethics committee.

Chapter 4:

- Dr Malinga Ratwatte assisted with retrospective data extraction and evaluation of RNFL measurements reliability.
- I have designed the study, performed all statistical analyses and written up the chapter.

Chapters 5, 6 and 7

- In collaboration with Dr Ian Smith and Professor Rupert Bourne I have designed these studies.
- I have secured a second external research grant (Fight for Sight, 15K).
- I have prepared ethical, HRA, Trust and NIHR Portfolio applications and studies' documentation.
- Jo Steel programmed the OpenClinica databases.
- I have screened all patients and organised the study visits.
- Hannah Munday provided administrative support.
- Dr Ian Smith and Professor Rupert Bourne helped with screening in sleep and glaucoma clinics respectively.
- Dr Catherine Willshire performed screening ophthalmic examination when required.
- I have recruited all patients and performed the relevant measurements. Dr Matthew Schneiders assisted with IOP measurements.
- I have extracted all data.
- I have liaised with the AngioVue development team to secure the latest version of analytical software for angiographic data processing prior to its commercial release.

- I have performed data analysis, including all statistical analyses. Dr Jules Hernandez-Sanches advised on sample size calculations.
- I have written the respective chapters.
- I have reported to the funders, the HRA and the Ethics committee.

# Chapter 3: Obstructive Sleep Apnoea in patients with Primary-Open Angle Glaucoma: prevalence and associations (the POSAG study).

(Prevalence of Obstructive Sleep Apnoea in Glaucoma)

## Null hypotheses:

i. The prevalence of OSA among patients with POAG is the same as in control subjects without glaucoma.
ii. There is no association between OSA severity markers and indicators of optic nerve structure and function in people with and without POAG.
iii. The STOP-BANG questionnaire cannot accurately identify POAG patients at high risk of OSA.

### 3.1 Introduction

The progressive and irreversible nature of vision loss in people with POAG, often despite adequate IOP lowering, necessitates careful assessment of other potential risk factors which can be modified in order to preserve the optic nerve function. As discussed in Chapter 1, the pathophysiological consequences of OSA at systemic and cellular levels are potentially relevant and, hypothetically, could promote glaucomatous optic neuropathy. For this reason screening for OSA has been recommended as part of the systematic work up in glaucoma (127,160,161). However, before any screening programme is implemented several factors need to be considered, including: the causality, the strength of association between the two conditions, the risks and benefits of a new diagnosis and the cost of additional diagnostic tests. One starting point would be to understand the burden of OSA among patients with glaucoma. At present, it is unclear whether people with POAG are more likely to suffer from OSA, are more symptomatic or have a different profile of OSA risk factors than those without glaucoma. A few small studies have assessed the prevalence of OSA in patients with POAG but they had important methodological limitations and have provided contradictory results(131,137,162,163).

Some authors have proposed the STOP-BANG questionnaire as a screening tool for OSA in those POAG patients who continue progressing despite adequately controlled IOP (160). Others used this questionnaire in epidemiological studies to evaluate the risk

for OSA among POAG patients (132). Because of its simplicity the STOP-BANG questionnaire is an attractive screening tool which could be potentially administered in busy glaucoma clinics. However, it was originally developed for screening of general surgical preoperative patients and has not been validated in people with POAG who have different demographics than the original derivative cohorts of surgical patients.

Several studies have shown excessive RNFL thinning and reduced retinal sensitivity in people with OSA and otherwise healthy eyes but these associations have not been examined in people with already established glaucoma and it is unclear how relevant these early changes are to the future risk of developing glaucoma (123–125).

The aim of the current study was, therefore, to determine the prevalence of OSA in a larger sample of patients with POAG, assess whether this prevalence is higher than in people without glaucoma with the same risk factors for OSA and whether it differs between POAG subtypes (HTG vs NTG). In addition, the study aimed to examine potential associations between OSA severity markers and indicators of optic nerve structure and function. The usefulness of the STOP-BANG questionnaire as a screening tool for OSA in patients with POAG is also assessed.

## 3.2 Methods

### *3.2.1 Study design and participants*

The study was observational and cross-sectional including unselected patients with POAG and control subjects. The project was approved by the East of England-Cambridgeshire and Hertfordshire Research Ethics Committee (REC number 15/EE/0292) and the Anglia Ruskin University Research Ethics Panel (ref. 15/16 014) (Appendix 5). Recruitment took place in the glaucoma clinic at a single secondary care hospital in the United Kingdom (Hinchingbrooke Hospital, North West Anglia Foundation Trust). Consecutive patients with a diagnosis of POAG who attended the clinic between March 2016 and September 2017 were invited provided they were historically able to perform reliable visual field tests. Control subjects with no history of glaucoma were recruited from spouses, partners, friends, and siblings of patients with POAG. There were two routes of approaching the potential control subjects: i. relatives of glaucoma patients who accompanied them in the glaucoma clinic were directly invited by the study personnel and provided written information outlining the study, ii. unaccompanied glaucoma patients were asked to pass on the study information with an invitation letter to their partners, friends or siblings who were then advised to contact the research team should they wish to contribute to the study. Participants had to be at least 18 years old and able to give informed consent. We invited all potentially eligible subjects irrespective of sleep complaints, including known SDB to participate.

*3.2.2 Study procedures*

Consented participants underwent the following assessments:

Baseline assessment

A brief medical history focused on cardiovascular, metabolic and ocular co-morbidities as well as medications was taken. Patients' records were reviewed to obtain details about POAG diagnosis, risk factors and management. Demographic and anthropomorphic variables, including: age, weight, height, BMI and neck circumference were collected and blood pressure was measured. The ESS and the STOP-BANG questionnaires were administered.

Ophthalmic examination

All enrolled participants underwent a comprehensive ophthalmic examination which consisted of the following measurements: best-corrected visual acuity, refraction (Auto kerato-refractometer, Topcon, Tokyo, Japan), automated visual fields (HFA, SITA-Fast, Carl Zeiss Meditec, Jena, Germany), slit-lamp biomicroscopy and gonioscopy (Haag-Streit International, Koeniz, Switzerland), Goldmann applanation tonometry (Haag-Streit International, Koeniz, Switzerland), CCT (Pachmate 2, DGH Technology Inc., Exton, USA) corneal hysteresis (Ocular Response Analyser, Reichert, N.Y., USA) and fundus photography. Circumpapillary RNFL thickness was automatically measured as a global average and also in four quadrants using Spectralis OCT (Heidelberg Engineering Inc. MA, USA).The global RNFL measurements were used in the analysis. The Mean Deviation (MD) and the Pattern Standard Deviation (PSD) from the visual field examination were used to categorize POAG patients into seven stages of glaucoma severity as per the enhanced Glaucoma Severity Staging system (eGSS) (164). Patients who had IOP≤21 mmHg prior to starting glaucoma treatment and during follow up constituted the NTG group. The diagnosis of POAG was confirmed by a consultant ophthalmologist specialising in glaucoma based on a typical optic disc appearance, reproducible visual field defect, and OCT results. Recruited POAG patients who did not meet the diagnostic criteria were excluded. Similarly, any control subjects found to have definite or suspected glaucoma were excluded.

For the analysis of associations between ocular and OSA parameters, the eye with the lower MD (greater visual field loss) was selected for each participant, unless it was affected by a comorbid condition which could have impacted visual field results or RNFL thickness. These comorbidities included: retinal vascular disease, diabetic retinopathy, age-related macular degeneration, prior retinal detachment, advanced cataract or neurological scotomas. Subjects with ocular comorbidity in both eyes or in the

glaucomatous eye in case of unilateral POAG, and also those who were using treatment for OSA, were excluded from the association analysis (Figure 3-1).

Sleep studies

Participants who met the eligibility criteria following the ocular assessment underwent nocturnal multichannel cardiorespiratory monitoring at home (rPG, type 3 sleep study) using a portable device (Embletta MPR and GOLD; Natus Medical Inc., Pleasanton, USA). The sleep study kit was set up during the study visit and it was demonstrated to the participants how to put it on. They were asked to return it on the following day with the enclosed questionnaire (Patient's Post Sleep Questionnaire) describing their sleep quality and estimated quantity on the sleep study night. Sleep studies of suboptimal quality or duration were repeated once; if the second attempt was unsuccessful or declined the participant was excluded. People using treatment for OSA were asked to withdraw it a week prior to the sleep test.

All sleep studies were then scored manually in the RSSC by one polysomnographer as described in the Methods Chapter. The polysomnographer was blinded to the glaucoma status. Participants with predominantly central sleep apnoea were excluded.

Individual follow up

All participants (and their General Practitioners) were subsequently informed of the outcomes of their sleep studies in writing. In addition, I conducted a telephone interview with all participants diagnosed with moderate to severe OSA and those who reported excessive sleepiness (ESS>10) irrespective of the severity of OSA to determine whether a formal assessment in the Sleep Disturbance Clinic for further investigation or treatment was needed.

### *3.2.3 Study outcomes*

Primary:

Difference between the groups in proportions of participants diagnosed with OSA (AHI≥5).

Secondary:

i. Difference between the groups in proportions of participants diagnosed with moderate to severe OSA (AHI≥15) and OSA syndrome (AHI≥5 +ESS>10).
ii. Differences in OSA prevalence between HTG and NTG patients.
iii. Associations between OSA severity (defined by AHI) and ocular parameters (functional visual impairment and RNFL thickness) in both groups.

iv. Predictive performance of the STOP-BANG questionnaire in discriminating between OSA vs No OSA (Area Under the Curve=AUC, sensitivity, specificity, predictive positive and predictive negative values for AHII≥5 and AHI≥15).

v. Differences in the rate of RNFL thinning in POAG patients with and without OSA. This outcome will be reported separately in Chapter 4.

### *3.2.4 Statistical Considerations*

#### *3.2.4.1 Sample Size*

The sample size was estimated based on the primary study objective of comparing the proportions of participants diagnosed with OSA in both groups. Data reported by Arnardottir et al. were used to estimate the prevalence of OSA in the control group (65). In that study the prevalence of OSA (defined as ≥5 AHI, type 3 sleep study) in the general Icelandic population with a mean age of 55 years was reported at 43%. Assuming the same prevalence of OSA in the UK population, a sample size of 232 participants per group would be required to detect a minimum difference of 13% in prevalence between the groups with 80% power and 5% significance (two-sided z-test for the difference between two independent proportions). A precision of 5% would be achieved with this sample size so that, for instance, if the OSA prevalence in the POAG group was 56% the 95% confidence interval would be between 51% and 61%. Larger differences could be detected with smaller sample sizes (see Appendix 6).

Based on these calculations and following feasibility assessment the study aimed to recruit 240 participants per group.

#### *3.2.4.2 Statistical analysis*

Continuous data are presented as mean (SD) or median (IQR) depending on the distribution. For all data, the assumption of normality was assessed using the Shapiro-Wilk test and by visual inspection of histograms. Independent continuous variables were compared using Student's t-test or, in case of non-parametric data, the Mann-Whitney U test. The chi-square test was used to compare proportions. In order to reduce confounding from imbalances between the groups in known OSA predictors, propensity score matching was performed using one-to-one nearest neighbour algorithm. The prevalence of OSA between POAG and control groups was then compared in the matched groups. I used an ordinal logistic regression model to assess for an association between POAG severity stages as the outcome measure and AHI as an independent variable. Univariate linear regression models were developed to examine the relationship between AHI and the global RNFL thickness. To assess whether OSA is a predictor of

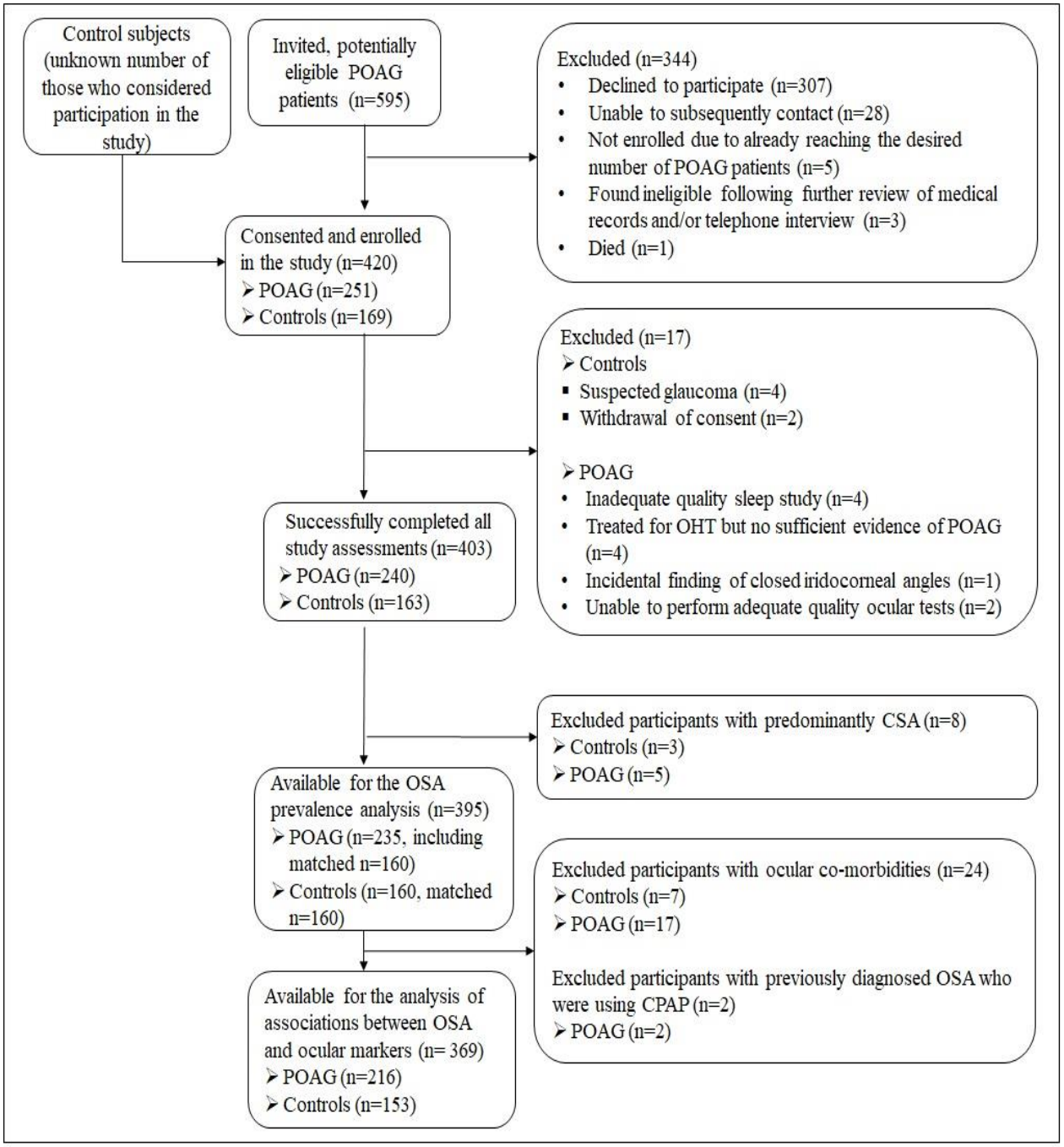
RNFL thickness after adjusting for previously reported predictors of glaucoma progression and/or RNFL thinning I built a multivariable linear model with AHI and other predictors as co-variates (forced entry method).

The area under the entire receiver operating characteristic curve (ROC AUC) was used to measure the overall discriminating ability of the STOP-BANG questionnaire in detecting OSA (separately for AHI ≥ 5 and AHI ≥15). The diagnostic performance (sensitivity, specificity, positive predictive values and negative predictive value) of the STOP-BANG questionnaire is reported at the conventional score of ≥3, and at the optimal cut-point defined by the Youden Index (*J*), which describes the maximum potential diagnostic effectiveness of the examined tool when equal weight is given to sensitivity and specificity (165).

All statistical test assumptions were adhered to throughout the analysis and only models meeting these assumptions are reported.

The propensity score matching was implemented in R (version 3.4.1) using the Matchit package. The ROC curves were constructed in MedCalc statistical software version 19.0.5 (Ostend, Belgium). All other analyses were conducted using SPSS software version 22.0 (IBM SPSS, IL, USA).

Figure 3-1. Flow diagram of the POSAG study.




Abbreviations: POAG=primary open angle glaucoma, OHT=ocular hypertension, OSA=obstructive sleep apnoea, CSA=central sleep apnoea, CPAP=continuous positive airway pressure.

## 3.3 Results

### *3.3.1 OSA prevalence*

A total of 395 participants was available for the OSA prevalence analysis comprising 235 with POAG and 160 control subjects (see Figure 3-1 for the study flow chart and Appendix 7 for demographic data on invited POAG patients who consented vs those who declined participation in the study). Spouses, partners, and friends of recruited POAG patients constituted 99% of the control group. OSA was diagnosed in 58% (95% CI:52-65) of POAG patients and in 54% (95% CI:47-62) of control participants (p=0.44, for between groups comparison). Moderate to severe OSA was found in 22% (95% CI:16-27) of POAG patients and in 16% (95% CI:11-22) controls (p=0.15, for between groups comparison). In the POAG group, five patients had been previously diagnosed with OSA. Two of them were using CPAP. One participant in the control group had a prior diagnosis of OSA and was not on treatment. In the entire cohort age, male sex, BMI, neck size and diabetes were significant predictors of OSA in unadjusted analyses (see Appendix 8). There were statistically significant (or close to being statistically significant) imbalances between the groups for sex and age, however, each of the participants in the control group was successfully matched with one of 160 POAG patients (Table 3-1).

There was no significant difference in OSA prevalence between the matched groups (53.8% [POAG] vs. 54.4% [Control], p=0.91). Similarly, the prevalence of moderate to severe OSA was not significantly different (18.1% [POAG] vs 16.3% [Control], p=0.66), Figure 3-2.

The level of sleepiness and the prevalence of OSA syndrome defined by a diagnosis of OSA and the presence of self-reported sleepiness (ESS score ≥11) did not differ between the participants with and without POAG (see Table 3-1).

Also, after excluding POAG patients with no measurable and mild visual field defects (stage 0, border and stage 1, according to the eGSS classification) there was no difference in the prevalence of OSA, and in OSA measures when the reminder of POAG patients were compared with the control group (see Appendix 9).

Data on baseline IOP prior to starting IOP lowering treatment were available in 92% of POAG patients. Of those, 35.6% were diagnosed with NTG and the remainder with HTG (see Appendix 10 for the baseline characteristic of HTG vs NTG patients). The prevalence of OSA was not significantly different between these two subgroups (55.8% [NTG] versus 57.6% [HTG], p=0.89). Moderate to severe OSA was diagnosed in 16.9 % of NTG patients and 24.5% of HTG cases (p=0.23). To account for statistically significant baseline differences in gender and BMI, a multivariable logistic regression model with OSA status (OSA vs no OSA) as a categorical dependent variable and sex, BMI and

POAG subgroup (HTG vs NTG) as independent variables was build. In this model, the POAG subgroup was not a significant predictor of OSA (p=0.81).

Table 3-1. Baseline Characteristics of Participants.

| | All POAG patients (n=235) | Control Group (n=160) | P value* | Matched POAG patients (n=160) | P value† |
|---|---|---|---|---|---|
| *Baseline characteristic of participants in relation to OSA* | | | | | |
| Male sex, n (%) | 135 (57) | 69 (43) | 0.005 | 73 (46) | 0.65 |
| Age, yr, (sd) | 70 (13) | 68.5 (11.8) | 0.056 | 69 (11) | 0.92 |
| White European, n (%) | 227 (97) | 156 (98) | 0.6 | 154 (96) | 0.52 |
| BMI, kg/m$^2$, (IQR) | 27.3 (5.2) | 27.3 (6.4) | 0.39 | 27.1 (5.6) | 0.52 |
| Neck size, cm, (IQR) | 40 (6.0) | 39 (5.9) | 0.11 | 39 (6.3) | 0.91 |
| Diabetes type 2, n (%) | 28 (12) | 13 (8) | 0.23 | 13 (8) | 1.0 |
| Hypertension, n (%) | 106 (45) | 74 (46) | 0.82 | 74 (46) | 1.0 |
| IHD, n (%) | 25 (11) | 15 (9) | 0.68 | 16 (10) | 0.85 |
| CVA, n (%) | 12 (5) | 7 (4) | 0.74 | 5 (3) | 0.56 |
| Atrial Fibrillation, n (%) | 14 (6) | 14 (9) | 0.29 | 11 (7) | 0.53 |
| AHI, per h, (IQR) | 6 (10.8) | 5.7 (9.7) | 0.26 | 5.2 (8.5) | 0.96 |
| ODI, per h, (IQR) | 4.1 (7.5) | 3.3 (7.1) | 0.29 | 3.7 (6.4) | 0.79 |
| T90, %, (IQR) | 17 (82) | 15 (76) | 0.48 | 15 (80) | 0.76 |
| MeanSpO2, %, (IQR) | 93 (1.9) | 92.8 (2.0) | 0.98 | 93 (2.0) | 0.72 |
| MinSpO2, %, (IQR) | 85 (6) | 86 (6.0) | 0.5 | 86 (6.0) | 0.73 |
| [#]Sleep Time, h, (IQR) | 7.3 (1.5) | 7.3 (1.3) | 0.55 | 7.3 (1.5) | 0.92 |
| [∞]Time in Bed, h, (IQR) | 7.6 (1.2) | 7.6 (1.4) | 0.42 | 7.6 (1.2) | 0.28 |
| ESS score, (IQR) | 6 (6) | 5 (6.0) | 0.92 | 5 (5) | 0.35 |
| ESS score ≥11, n (%) | 31 (13) | 26 (16) | 0.4 | 16 (10) | 0.1 |
| OSAS, n (%) | 19 (8) | 14 (9) | 0.84 | 10 (6) | 0.4 |
| STOP-Bang score, (IQR) | 4 (3) | 3 (3) | 0.19 | 3 (3) | 0.8 |
| *Baseline characteristic of participants in relation to ocular parameters*[&] | | | | | |
| MD, dB, (IQR) | -3.6 (6.7) | -0.7 (2.1) | 0.00 | -3.3 (6.9) | 0.00 |
| VFI, %, (IQR) | 93 (18) | 99 (1.0) | 0.00 | 93 (18) | 0.00 |
| cRNFL, µm, (sd) | 68.6 (15.8) | 93.9 (11.1) | 0.00 | 68.6 (15.8) | 0.00 |
| CDR, (IQR) | 0.8 (0.3) | 0.4 (0.2) | 0.00 | 0.8 (0.3) | 0.00 |

*For the difference between All POAG patients and Control Group

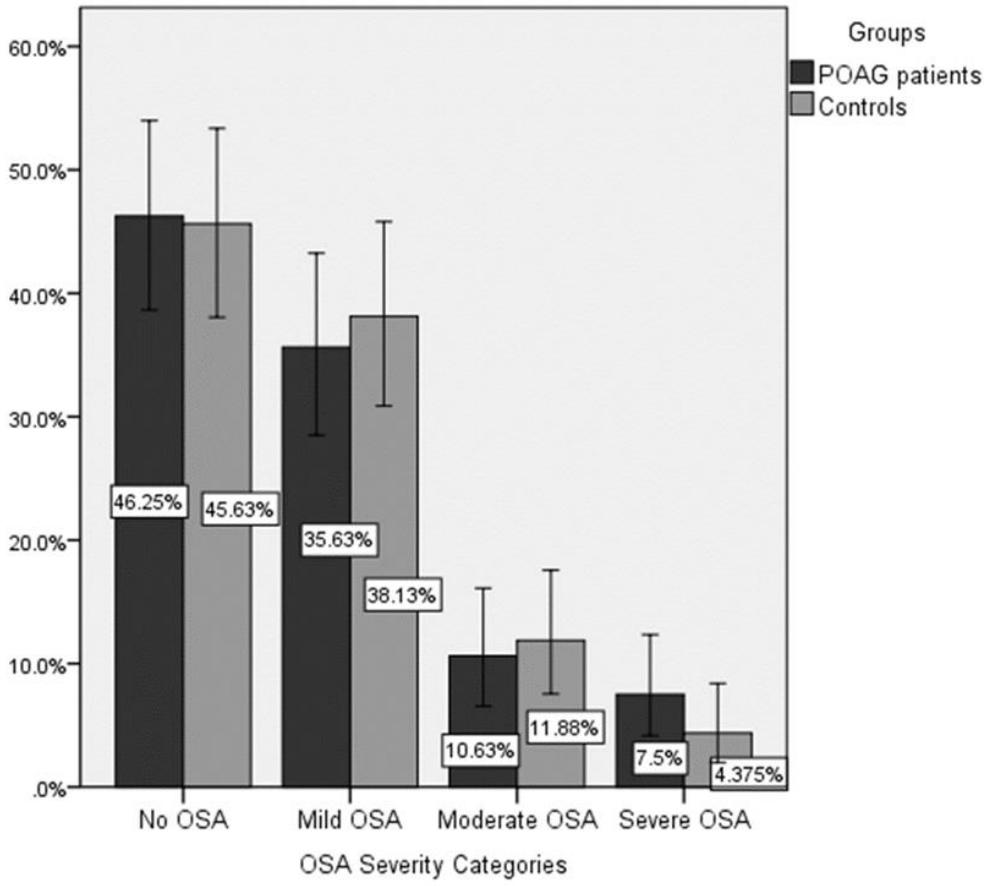
†For the difference between Matched POAG patients and Control Group

[#]Self-reported estimated sleep time

[∞]Device recorded time in bed.

[&] Based on data from both eyesDefinition of abbreviations: BMI=body mass index, IHD=ischaemic heart disease, CVA=cerebrovascular accident, AHI=apnoea-hypopnoea index (the measurement of reduction and cessation of airflow per hour), ODI=oxygen desaturation index ( the number of 4% oxygen dip per hour), T90=time spent with oxygen saturation less than 90%, MeanSpO2=mean oxygen saturation, MinSpO2=minimum oxygen saturation, ESS=Epworth Sleepiness Scale, OSAS=obstructive sleep apnoea syndrome (AHI≥5 and ESS1≥11), STOP-Bang is a questionnaire used to evaluate the likelihood of OSA. It consists of questions about: snoring, tiredness/sleepiness/fatigue, witnessed apnoeas, hypertension, BMI, age, neck circumference and gender. The minimum score is 0 and the maximum 8, MD=mean deviation, VFI=visual field index, cRNFL=circumpapillary retinal nerve fibre layer thickness, CDR= cup to disc ratio (determined by the optometrist based on a slit lamp examination).

Figure 3-2. Distribution of participants in the matched POAG and the control group according to OSA severity.




Definition of abbreviations: POAG=primary open-angle glaucoma, OSA=obstructive sleep apnoea.

*3.3.2 Associations between OSA and ocular parameters*

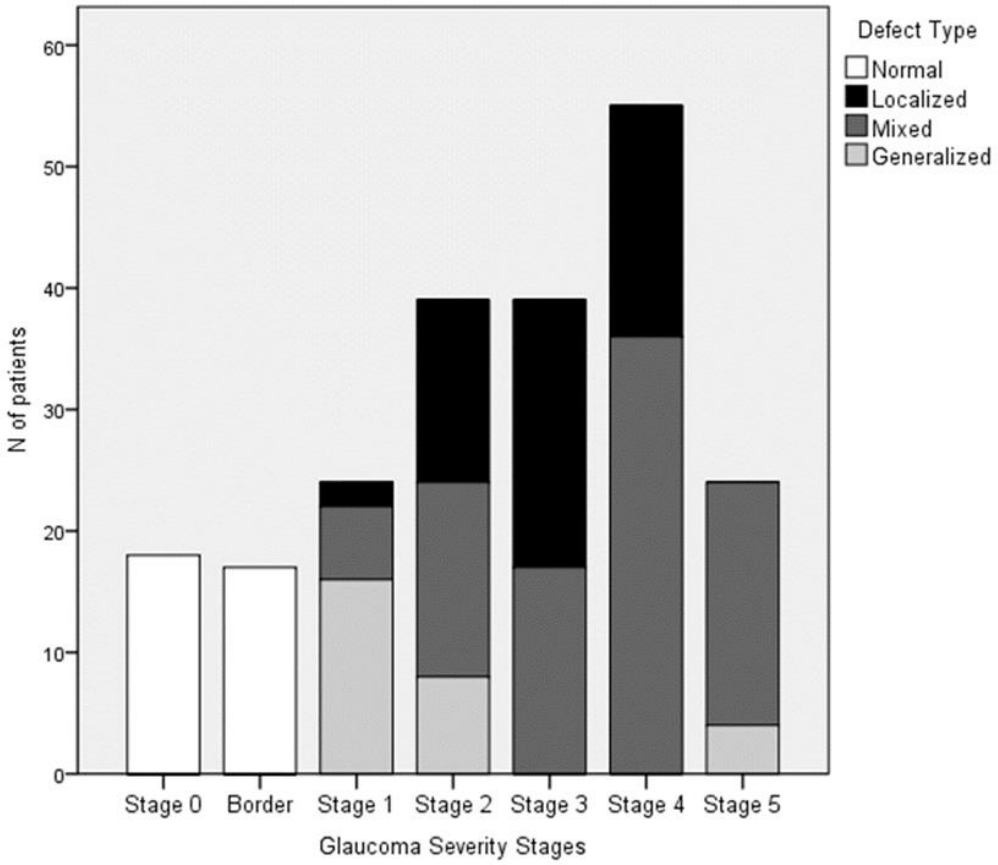
After exclusion of participants with treated OSA and ocular co-morbidities, data from 216 POAG patients and 153 control subjects were available for the association analysis.

The POAG group consisted of patients with all stages of functional visual field impairment (Figure 3-3). According to the chosen classification, Stage 0 represents no detectable visual field defect and the higher the stage the more severe the visual field loss.

All visual field tests were reliable (false negative errors <20%, false positive errors <20%, fixation loses<33%) and there were no missing data. In an ordinal logistic regression analysis, AHI was not a significant predictor of the disease severity stages (p=0.40), Figure 3-4. To assess whether there was a relationship between AHI and the structural damage I used linear regression models. Subjects with missing data or inadequate quality images were not included in the models (for further details see Appendix 9). The AHI did not predict RNFL thinning in an unadjusted model and remained non-significant in a multivariable model. Highest ever recorded IOP, IOP measured at the study visit, and corneal hysteresis were independently associated with RNFL thickness. I constructed similar linear regression models in the control group and found no significant associations between AHI and RNFL thickness. In a multivariable model, older age, male sex and higher degree of myopia were the only independent predictors of RNFL thinning (Table 3-2).

Furthermore, there was no difference in the global RNFL thickness between people with and without OSA in both groups. The global RNFL thickness did not differ even when participants without OSA were compared with those with moderate to severe OSA (Table 3-3).

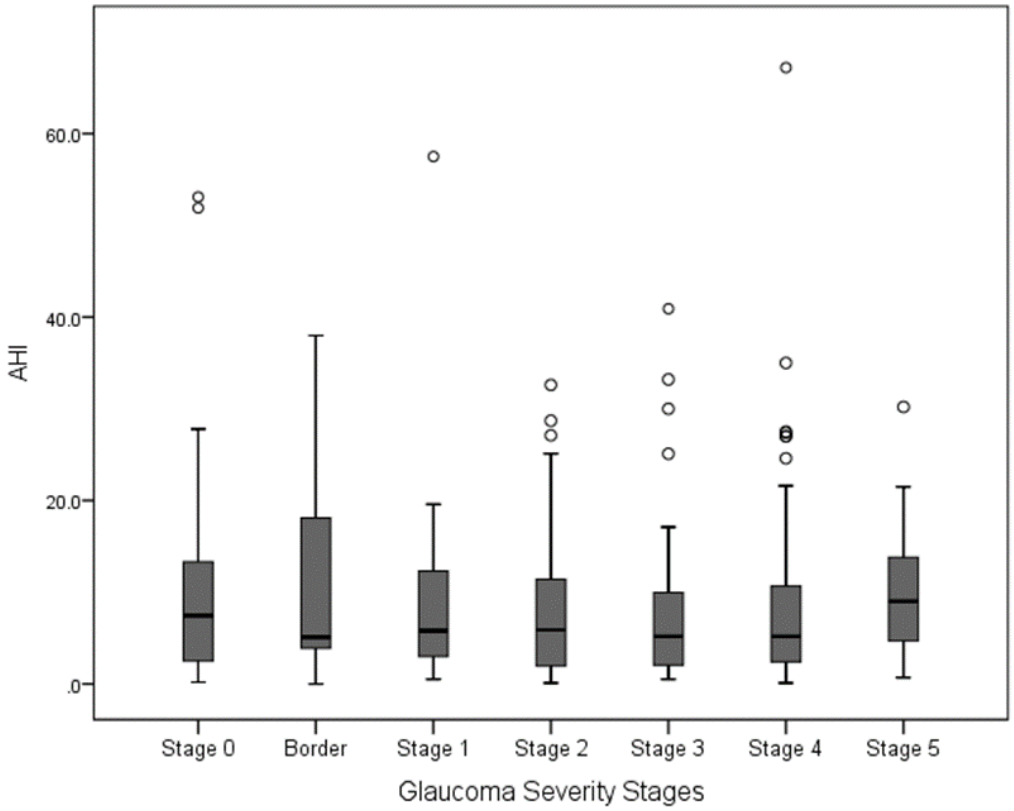
Figure 3-3. Severity of functional damage among recruited POAG patients (unmatched POAG group) based on the Enhanced Glaucoma Staging System (eGSS). For each patient the eye with the lower MD was selected.




Mean Deviation (MD) (median ±IQR) for each POAG severity stage:

Stage 0: -1.1±0.77 dB, Border: -1.7±0.66 dB, Stage 1: -3.1±0.93 dB, Stage 2: -4.5±1.92 dB, Stage 3: -7.3±2.1dB, Stage 4: -11.9±3.9 dB, Stage 5: -21.3±4.7 dB.

Figure 3-4. Distribution of AHI across POAG severity stages (Error bars represent 95% CI). Data analysed in an unmatched POAG group.




Definition of abbreviations: AHI=apnoea-hypopnea index,

POAG=primary-open angle glaucoma.

Table 3-2. Summary of the linear regression models for the associations between AHI and global RNFL thickness. All models were constructed in unmatched groups following exclusions described in Appendix 11.

| Model | N | Adjusted $R^2$ | Beta | t value | P value | 95% CI |
|---|---|---|---|---|---|---|
| POAG | | | | | | |
| Unadjusted: AHI | 202 | 0.004 | 0.095 | 1.4 | 0.18 | -1.6 to 8.5 |
| Fully adjusted: | 189 | 0.16 | | | 0.000 | |
| AHI | | | 0.003 | 0.41 | 0.97 | -0.18 to 0.19 |
| IOPpeak | | | -0.23 | -3.2 | 0.002 | -0.77 to -0.18 |
| IOPcurrent | | | 0.29 | 3.9 | 0.000 | 0.48 to 1.5 |
| Hysteresis | | | 0.24 | 3.0 | 0.003 | 0.55 to 2.6 |
| CVS | | | -0.13 | -1.9 | 0.061 | -8.10 to 0.19 |
| Male sex | | | -0.12 | -1.8 | 0.074 | -3.50 to 7.6 |
| Age | | | 0.011 | 0.144 | 0.9 | -0.23 to 0.26 |
| CCT | | | 0.048 | 0.65 | 0.52 | -0.04 to 0.08 |
| SER | | | 0.058 | 0.77 | 0.44 | -0.46 to 1.1 |
| Controls | | | | | | |
| Unadjusted: AHI | 146 | .008 | -0.12 | -1.4 | 0.15 | -7.4 to 1.1 |
| Fully adjusted: | 131 | 0.2 | | | 0.000 | |
| AHI | | | 0.01 | 0.13 | 0.9 | -0.14 to 0.16 |
| SER | | | 0.34 | 4.4 | 0.000 | 0.9 to 2.4 |
| Male sex | | | -0.27 | -3.4 | 0.001 | -9.2 to -2.4 |
| Age | | | -0.16 | -2.1 | 0.041 | -3.8 to -0.01 |

Definition of abbreviations: RNFL=retinal nerve fibre layer, AHI=apnoea-hypopnea index, N=number of subjects in the model (subjects without missing data), CI=confidence interval, AHI=apnoea-hypopnea index, IOPpeak=highest ever recorded intraocular pressure, IOPcurrent=intraocular pressure recorded at the study visit, SER= Spherical Equivalent Refraction, CCT=central corneal thickness, CVS=cardiovascular co-morbidities (at least one of the following: diabetes, hypertension, ischemic heart disease, atrial fibrillation, cerebro-vascular accident, heart failure, over 20 pack year smoking history.

Table 3-3. Retinal Nerve Fibre Layer thickness according to OSA status. Data analysed in unmatched groups following exclusions described in Appendix 11.

| | No OSA | OSA | P value* | Moderate to Severe OSA | P value† |
|---|---|---|---|---|---|
| POAG group<br>Global RNFL (μm) | 62.6 (14.7) | 63.9 (14.3) | 0.54 | 65.2 (15.2) | 0.37 |
| Control group<br>Global RNFL (μm) | 94.8 (11.1) | 92.5 (10.5) | 0.2 | 94.8 (9.9) | 0.99 |

*For the difference between No OSA (AHI<5) and OSA (AHI≥5).

† For the difference between No OSA (AHI<5) and moderate to severe OSA (AHI≥15).

Definition of abbreviations: OSA=obstructive sleep apnoea, RNFL= Retinal Nerve Fibre Layer.

### *3.3.3 Assessment of the STOP-BANG questionnaire in detecting OSA in POAG patients and controls.*

The diagnostic performance of the STOP-BANG questionnaire was first analysed in the matched POAG group. Of the 160 POAG patients 109 (68%) had scores of ≥3 (Figure 3-5). The AUC in the matched POAG group for predicting the diagnosis of OSA (AHI≥5) was only 0.64 (95% CI: 0.56–0.71, p=0.001). The Youden's index indicated an optimal cut off point at the score of ≥3 but the associated sensitivity, specificity, positive predictive values and negative predictive value were low: 58%, 66%, 67% and 58%, respectively (also see Appendix 12). The questionnaire had no predictive value for moderate to severe OSA (AUC=0.56, p=0.30, 95% CI: 0.48-0.64). In the whole cohort of POAG patients (n=235) the AUC for the diagnosis of OSA also indicated poor discriminative value (AUC=0.61, p=0.004, 95% CI 0.54 to 0.67) and no discriminative value for predicting moderate to severe OSA (AUC=0.54, p=0.32, 95% CI: 0.48 to 0.61).

The questionnaire performed better in the control group but still had only fair discriminative ability (AUC=0.75, p<0.001, 95% CI: 0.68 to 0.82 for AHI≥5 and AUC=0.78, p<0.001, 95% CI: 0.71 to 0.85 for AHI≥15). The differences between the matched groups in AUC were statistically significant (ΔAUC=0.112, p=0.049 for AHI≥5 and ΔAUC=0.223, p=0.003 for AHI≥15) (Figure 3-6).

Figure 3-5. Distribution of STOP-BANG scores in matched POAG patients and proportions of patients diagnosed with obstructive sleep apnoea (OSA).

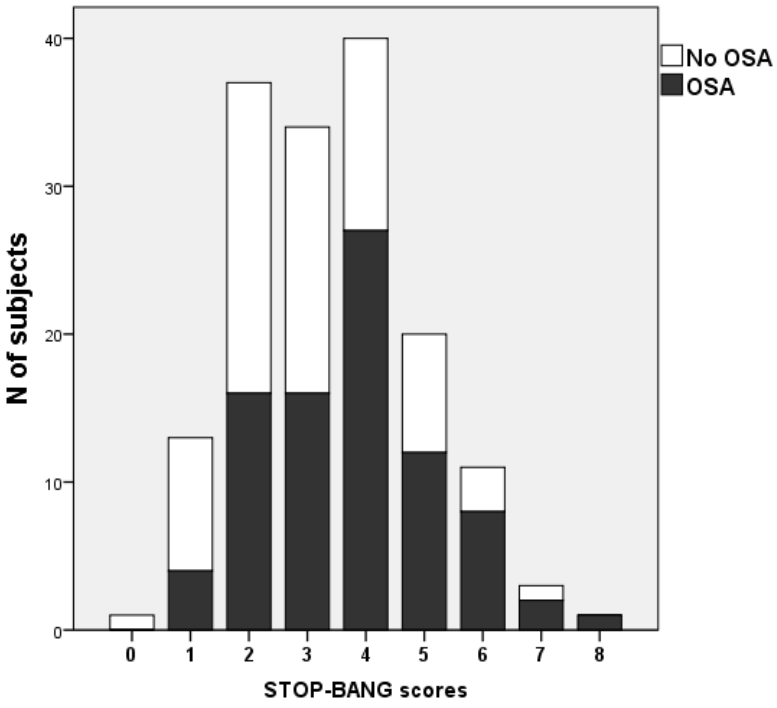
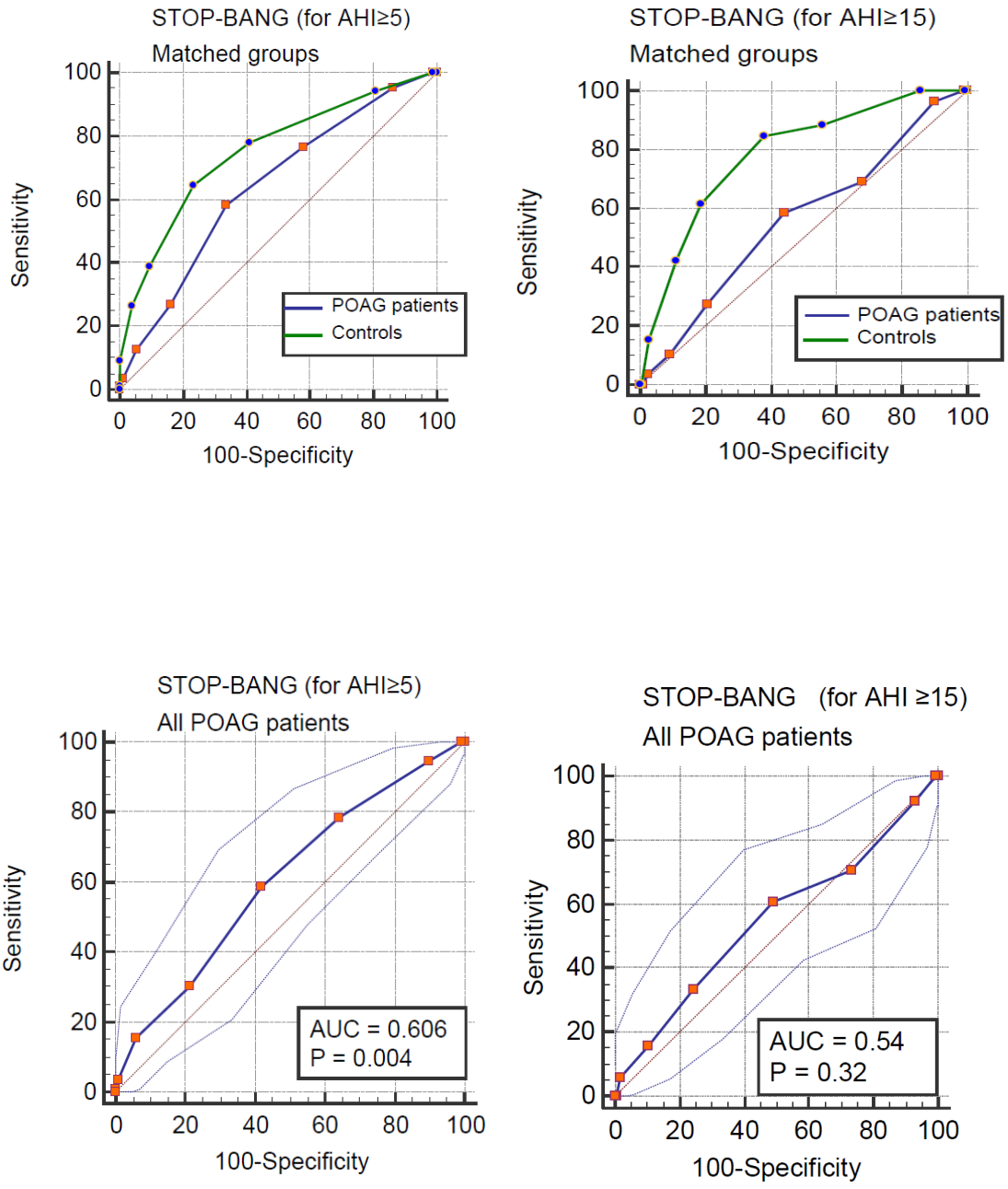


Figure 3-6. Receiver operating curves (ROC) for STOP-Bang to predict AHI≥5 and AHI≥15 in matched groups (top graphs) and in all POAG patients (bottom graphs).



A dissection of the STOP-BANG questionnaire items in relation to the distribution of these variables in the examined matched cohorts of participants and their individual predictive values sheds some light on the potential roots of the overall poor predictive performance of the questionnaire, particularly in the POAG group. For instance, the questionnaire uses age cut off >50 years. Only 2.5% of POAG patients 3.8% of controls were below this age, all others scored one point on this item. The threshold for BMI is set at >35kg/m2. Only 3.8% of POAG patients and 9.4% of the control subjects reached this threshold. Also, a logistic regression analysis run separately in each group indicates that, in contrast to expectations, male sex was not a significant predictor of OSA in either group (POAG: OR 1.6, 95% CI: 0.87 to 3.1, p=0.13, Controls: OR 1.77, 95% CI: 0.93 to 3.3, p=0.08) and hypertension was associated with OSA only in the control group (POAG: OR 1.54, 95% CI: 0.82 to 2.88, p=0.18, Controls: OR 3.44, 95% CI: 1.78 to 6.65, p=0.000). Therefore, at least 4 items of this questionnaire in the POAG cohort and 3 in the control group were probably of limited contribution to its overall predictive ability.

## 3.4 Discussion

### *3.4.1 OSA prevalence*

Based on plausible pathophysiological mechanisms and some epidemiological data it has been hypothesised that OSA may play a role in the development and progression of glaucomatous optic nerve neuropathy (121,161,166,167). According to this hypothesis, a high OSA burden could be expected among glaucoma patients and there would be a rational to evaluate screening for OSA. In the current study of unselected POAG patients prospectively examined with a diagnostic test for SDB in a real-world clinic scenario, I found no evidence that patients with POAG are more likely to suffer from OSA or have more severe OSA than control subjects. After statistical matching of participants with and without glaucoma, the prevalence of OSA was remarkably close between the groups. In addition, I have found no difference between the groups in several markers of nocturnal hypoxia associated with OSA and the levels of subjective sleepiness (when considered on its own or in a combination with OSA diagnosis as OSA syndrome) were similar. This indicates that if routine OSA screening was to be implemented in glaucoma clinics the yield of patients who would then be considered for OSA treatment, according to the current clinical practice, would be the same as in the general population of middle to elderly aged people. The reported prevalence of OSA (defined as ≥5 AHI) in the general adult population varies widely from 9 % to 38% (68). There are multiple reasons for these differences across the studies. Of particular importance are population specific factors, such as: age, ethnicity and the prevalence of obesity. There are also factors related to

OSA measurement techniques including the type of sleep study used and the scoring criteria for respiratory events which have changed several times in the last two decades. This highlights the importance of having a well matched concurrent control group and using the same diagnostic tools whenever OSA prevalence is studied in disease-specific cohorts of patients.

Although the OSA prevalence figures found in the current study in both groups seem high they are in keeping with relatively high rates of OSA reported in older people. For instance, in a study from Iceland OSA (AHI ≥5) was found in 43.1% of participants aged 40-65 years who were also examined with type 3 sleep study (65). In a slightly older (mean age of 68 years) French cohort, 57% of participants were diagnosed with OSA (AHI ≥15) based on the same type of sleep study(168). The largest to date population based study conducted in Switzerland which used a more sensitive type 2 sleep study (unsupervised full polysomnography) found OSA (AHI ≥5) in 84% of people aged 60-85 years (169).

Previous prospective studies assessed the prevalence of OSA among POAG patients but had important methodological limitations related to small sample sizes, pre-selecting patients based on symptoms, including historic control groups, using only low-grade sleep tests or indeed just questionnaires to assess for possible OSA (131–133,137,162). Most of these studies suggested that OSA may be more common in patients with POAG but Roberts et al. who performed nocturnal oximetry on the largest previously published sample (52 POAG patients and 60 control subjects) found no significant differences in SDB prevalence. In their study, 17% of POAG subjects and 12% of controls were diagnosed with SDB based on a 4% oxygen desaturation index (ODI) above 20. The current study has the added value of a larger sample size, multichannel sleep tests which allowed me to assess SDB more accurately and a concurrent control group who had glaucoma excluded based on a detailed ocular examination.

Although, it has been suspected that OSA may be particularly relevant in NTG where retinal ganglion cell apoptosis seems less IOP dependent, in this study the prevalence rates of OSA were not different when patients with NTG and HTG were compared. I have found no previous studies which directly compared OSA burden between patients with NTG and HTG.

### *3.4.2 AHI and RNFL thickness*

To further explore the relationship between OSA and POAG I examined potential associations between AHI and RNFL thickness. Despite adjusting for factors which were likely to have a dominant effect on RNFL thickness, including IOP, I have not found AHI to be a predictor of RNFL thinning in POAG patients or in the control group. Furthermore, the RNFL thickness was similar when people with moderate to severe OSA were compared with participants without OSA separately in both groups. Previous studies have assessed RNFL thickness in relation to OSA in people with otherwise healthy eyes. Most reported thinner RNFL in people with OSA as summarized in three recent meta-analyses (170–172). The relationship between AHI, or other markers of OSA, and RNFL was rarely examined in the source papers for these meta-analyses, and when it was the models were not adjusted for other important RNFL predictors (173,174). Nevertheless, the findings of the current study cannot be simply compared with the previous studies due to a different cohort of subjects in our control group. In contrast with the previous studies which exclusively recruited people who presented to sleep centres with OSA symptoms (and therefore may have represented enriched samples of patients also at higher risk of systemic effects of OSA), the control group in this study consisted of a population-based sample of largely asymptomatic participants screened for OSA. Thus, it is possible that patients with only certain OSA phenotypes are at risk of aggravated RNFL loss. It is also noteworthy that the control subjects were older than in most previous studies. As RNFL thickness declines with age and older people are more likely to suffer from hypertension and atherosclerosis which can also potentially affect RNFL, it may have been more difficult to appreciate the impact of OSA in the current control group.

### *3.4.3 STOP-BANG questionnaire as a screening tool for OSA in POAG patients*

When validated against a type 3 sleep study STOP-BANG scores did not discriminate well between the presence or absence of OSA in patients with POAG. This is evidenced by the low AUC and the lack of a cut off score with acceptable sensitivity and specificity. Any screening tool used to risk stratify patients to those who require further investigations and those in whom a condition can be confidently ruled out must have a high sensitivity. A STOP-BANG score of 3 and above is considered to represent a higher risk of OSA prompting further evaluation with a sleep study. In the original validation study of surgical patients this cut-point had a sensitivity of 84% for AHI≥5 and 93% for AHI≥15 (142). Surprisingly, in the current study the questionnaire performed better for the lower rather than the higher AHI cut off in POAG patients. However, it still had unacceptably low

sensitivity of 58% for the score of 3 which would give false reassurance to a substantial proportion of patients that they do not require further investigations. With higher scores the sensitivity fell dramatically. Moving the cut-point to 2 increased the sensitivity only to 77% and, irrespective of this, would not be helpful in the examined population as the vast majority of patients had a score of at least 2. For instance, with a lower threshold nearly all male patients with POAG would be deemed at high risk of OSA based on sex and age alone.

An older age, lower BMI and high prevalence of hypertension in the examined cohorts of patients in the current study compared to the surgical population could explain the worse predictive performance of the STOP-BANG questionnaire. Thus, recalibration of the questionnaire by either changing the cut offs for some items or reweighting the scores based on odds ratios estimated by logistic regression analysis could be attempted to align it with the characteristics of the examined population. However, this may not be sufficient to improve the diagnostic accuracy of the scores in the POAG group. The reasons behind significantly worse discriminative ability of STOP-BANG in POAG patients compared to matched controls are not entirely clear and may be related to other inherent risk factors not captured by the questionnaire. On the contrary, items which have been shown to predict the risk of OSA in other cohorts may have no predictive value in POAG patients. For instance, I cannot explain why hypertension was significantly associated with OSA in the control group but not in matched and unmatched POAG groups. A hypothesis that systemic hypertension in POAG patients is not driven or contributed by OSA could be formulated and set another direction for future research.

### *3.4.4 Limitations*

The main limitation of this study is its cross-sectional design. Although we have recruited a representative sample of patients with all stages of glaucoma severity I cannot rule out the possibility that OSA contributes to POAG progression. OSA is not consistently present throughout the person's lifetime but develops largely as a function of weight gain and aging. Therefore, whilst it is unlikely that OSA is a potent enough risk factor to alone cause glaucomatous optic neuropathy, particularly in people who had it for a relatively short period of time, it is still possible that it may promote the neural damage in those who have already developed glaucoma as a result of other risk factors. This can be determined only in a longitudinal study.

We invited all consecutive glaucoma patients without prior knowledge of their sleep history but, since 58% of those invited declined study participation, I cannot exclude

selection bias. Further, because of the nature of our recruitment pathways in the control group I could not ascertain the proportion of potentially eligible subjects who were invited via the glaucoma patients but declined to take part in the study. Therefore, it was not possible to compare the actual recruitment rates between the groups. It could be argued that glaucoma patients were more likely to take part in the study even though they had no sleep related symptoms because of the long-term relationship with the health care provider and perceived benefits of contributing to a research into the disease which affected them. On the contrary, the control subjects may have been incentivised to enrol only if they had relevant sleep complaints. This could lead to underestimation of OSA prevalence in the POAG group and its overestimation in the control group. However, against this hypothesis there are no differences detected between the groups in the ESS which assesses subjective sleepiness, a cardinal symptom of OSA, and the STOP-BANG questionnaire which incorporates questions about snoring, tiredness and witnessed apnoeas. It is also worth noting that spouses of glaucoma patients who took part in this study constituted 85% (spouses and friends-99%) of the control group and there were no recruits among relatives of those glaucoma patients who declined study participation. This indicates that glaucoma patients and their spouses were willing to contribute to this research as couples and this may have helped to reduce the risk of selection bias at least for the between group comparisons. If the selection bias did occur, most likely it would have affected the OSA prevalence rates in both groups in the same direction, although I cannot exclude other possible scenarios.

The study recruited the originally planned number of POAG patients but under-recruited control subjects. The decision to terminate the study after reaching the desired number of POAG patients was based on minimal differences in OSA prevalence observed between the groups with largely overlapping confidence intervals and no indication from a simulation analysis that increasing the precision of the estimated parameter by recruiting a larger sample of controls would alter the significance of the comparisons. Even with a smaller sample of control subjects this is still the largest reported study of its kind.

Finally, as over 90% of recruited patients were of white European origin the findings of this study are not generalisable to other ethnic backgrounds.

### 3.5 Conclusions

In conclusion, based on the prevalence analysis and assessment of associations between OSA and POAG markers this study found no evidence to support the hypothesis that OSA causes glaucoma. In light of these findings the systematic screening of POAG patients for OSA cannot be recommended. The STOP-BANG questionnaire has poor diagnostic accuracy to detect OSA in these patients and in its current form should not be used as a screening tool. Future longitudinal studies should address the question of whether untreated OSA is associated with faster rates of glaucoma progression. The next Chapter is dedicated to examining this problem.

# Chapter 4: Is OSA a risk factor for reduction of Retinal Nerve Fibre Layer Thickness in Primary Open-Angle Glaucoma? Longitudinal data.

## Null hypothesis:

iv. A longitudinal change in RNFL thickness in POAG patients with OSA is the same as in those without OSA.

### 4.1 Introduction

The retinal nerve fibre layer (RNFL) is the innermost layer of the retina. It is formed by the retinal ganglion cell axons which connect the outer neuroretina to the dorsal lateral geniculate nucleus of the thalamus(175). Repeated RNFL evaluation is currently an integral part of glaucoma monitoring. It allows for an early detection of the disease and its progression, often several years before the corresponding visual field loss ensues. Peripapillary RNFL thinning observed over time represents progressive loss of retinal ganglion cell axons and can be quantitatively measured with several different techniques currently available. Of these, the spectral-domain optical coherence tomography (SD-OCT) is probably the most precise (176). The structural OCT measurements are highly reproducible, relatively easy to obtain and, unlike visual field tests, do not rely on subjective responses of the patient (177,178). These characteristics make OCT a useful tool in examining the impact of OSA on glaucoma and potentially other neurodegenerative disorders such as Alzheimer's disease which also affect the optic nerve (179). Cerebrovascular insufficiency, hypoxia and oxidative stress associated with OSA may contribute to premature and accelerated degeneration of retinal ganglion cells which due to their high metabolic activity are particularly sensitive to oxygen and energy depletion(53). Loss of retinal ganglion cells and their axons would lead to the structural changes detected by the OCT.

As discussed in Chapter 3, several previous cross-sectional studies which examined RNFL thickness in OSA patients without glaucoma reported contradictory findings. Some of them found thinner RNFL in OSA subjects while others, including the study described in the previous Chapter, showed no relationship between RNFL thickness and OSA status or AHI (174,180–188). Differences between the studies in terms of demographics of recruited participants, the RNFL measurement techniques and whether or not other factors known to affect RNFL thickness were accounted for could partly explain the discordant outcomes. Nonetheless, the major limitation of cross-sectional studies is that

they only offer a snapshot of a single moment in the disease process without considering the temporal changes in the examined variables. In the case of OSA, one of the main challenges is that it is difficult to determine how long the individual patient has been affected by it. In addition to OSA severity, its duration, that is the exposure time to chronic intermittent hypoxia and other pathophysiological consequences of interrupted nocturnal ventilation, is likely to be an important determinant of its potential to cause end-organ damage. In terms of POAG, one-off measurements of RNFL may simply represent the end product of previously elevated IOP making it difficult to detect less potent risk factors. Longitudinal studies which measure changes in RNFL thickness over a period of time which is relatively close to a diagnostic sleep test and in patients previously established on hypotensive treatment to reduce the confounding effect of IOP would stand a better chance of more accurately assessing for the hypothesised contribution of OSA to the structural optic nerve damage.

The purpose of this Chapter is to examine whether POAG patients with untreated OSA have greater loss of RNFL during the monitoring period than those without OSA.

## 4.2 Methods

### *4.2.1 Study design and procedures*

This was a retrospective analysis of longitudinal OCT data acquired as part of a routine follow up in the Glaucoma Clinic of POAG patients who took part in the POSAG study and underwent a prospective diagnostic sleep test for OSA as well as a comprehensive ocular examination described in Chapter 3. All 240 POAG patients who had successfully completed the POSAG study were screened for eligibility to this analysis.

The following enrolment criteria were applied:

*Inclusion criteria*

1. A minimum of 3 years of OCT follow up data at the point of data extraction which took place approximately 6 months after recruitment to the POSAG study had finished.
2. OCT scans of adequate quality. The image quality was assessed at the point of data extraction. Images with RNFL segmentation errors, inaccurate optic disc margins delineation, low signal strength and artifacts were excluded.

*Exclusion criteria*

1. Patients diagnosed with central sleep apnoea.
2. Patients who received treatment for OSA.
3. Eyes with co-morbidities which could affect RNFL thickness, including: retinal vascular disease, diabetic retinopathy, age-related macular degeneration, prior retinal detachment, advanced cataract or neurological scotomas.

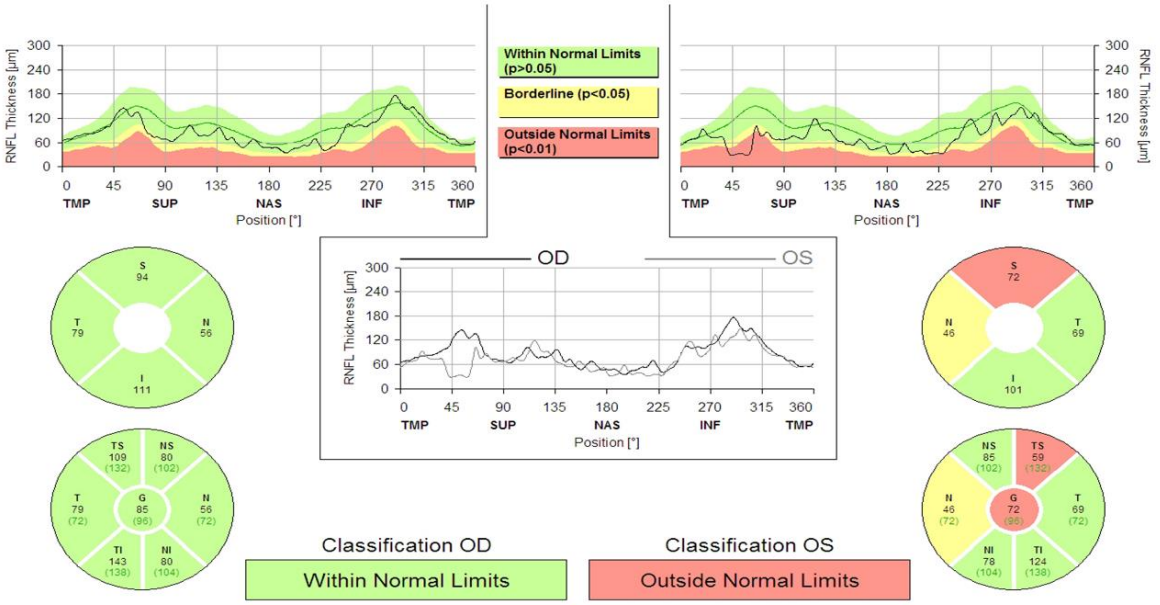
For each patient, both eyes were included in the analysis provided they individually satisfied the eligibility criteria.

All scans were acquired using Spectralis OCT (Heidelberg Engineering Inc. MA, USA), an SD-OCT scanner described in Chapter 2. This device was first introduced in the Glaucoma Clinic approximately 6 years before recruitment to the POSAG study started and gradually replaced the HRT (Heidelberg Retina Tomograph) device previously used for structural assessment of the optic nerve. Therefore, for the majority of patients less than 3 years of OCT follow up data were available. However, a minimum of 3 years of observation was thought to be required to detect measurable RNFL changes which could be attributed to glaucoma progression. This is in keeping with a monitoring period selected in other studies which investigated structural glaucoma progression (189–191). Data on global and quadrant specific (superior, temporal, inferior and nasal) circumpapillary RNFL thickness were extracted from the first and the last available adequate quality scans. Progression was determined by RNFL thickness change over the monitoring period and was calculated as the difference in RNFL thickness between the most recent and the first available scans divided by the number of follow up years ($RNFL_{last\ [\mu m]}$-$RNFL_{baseline\ [\mu m]}$/years) (see Figure 4-1 for an example of Spectralis OCT output for a patient with progressive RNFL loss).

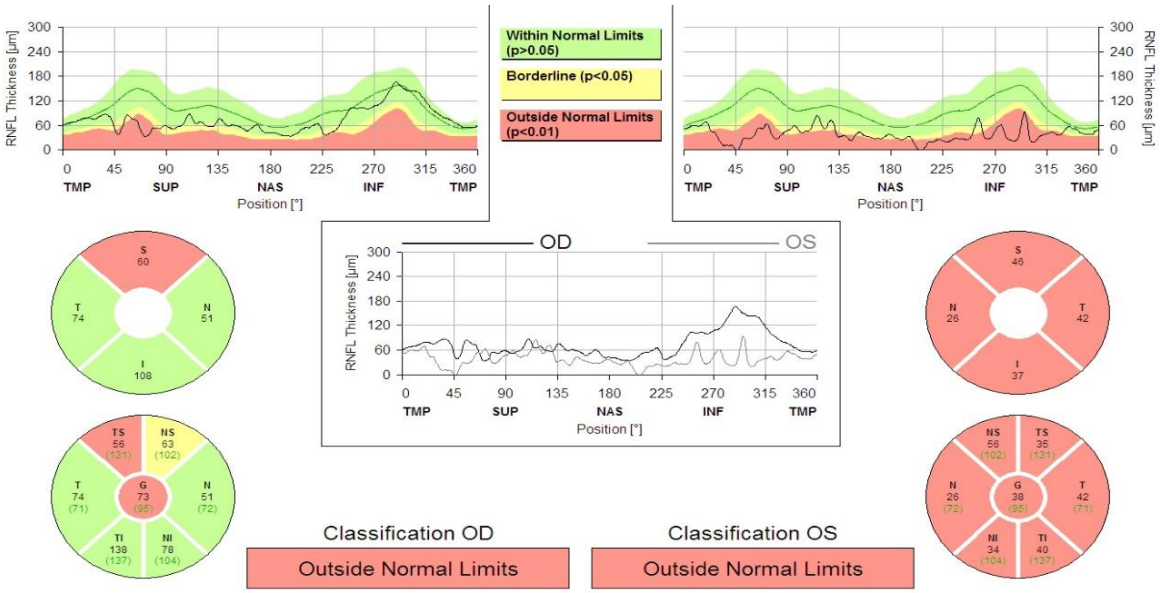
This analysis was performed under the ethical approvals granted for the POSAG study (East of England-Cambridgeshire and Hertfordshire Research Ethics Committee; REC number 15/EE/0292 and the Anglia Ruskin University Research Ethics Panel; ref. 15/16 014, Appendix 5).

Figure 4-1. Example of peripapillary OCT scans demonstrating progressive RNFL loss in a POAG patient with moderate OSA (AHI=27).

First scan: 27.03.2012




Last scan: 08.01.2018



*4.2.2 Statistical analysis*

All analyses were conducted using SPSS software version 22.0 (IBM SPSS, IL, USA). The level of significance was set at $p < 0.05$. Baseline continuous data are presented as mean (SD) or median (IQR) depending on the distribution. Categorical data are reported as percentages. Comparisons of clinical characteristics between the groups, with the exception of ophthalmic variables, were performed using independent-samples T-test or, if the data were not normally or approximately normally distributed, and in case of categorical variables, using the Mann-Whitney U test.

For ophthalmic data analysis the generalized estimating equation (GEE) method was used to account for correlation between the two eyes of the same patient. To establish whether OSA status (OSA vs no OSA) is a significant predictor of RNFL thickness change a univariate linear regression model was developed within the framework of GEE. This model was subsequently adjusted for variables previously associated with RNFL loss, including: age, IOP, cardiovascular co-morbidities, baseline RNFL thickness, previous filtration surgery and corneal hysteresis (190,192–197).

The GEE method is a population-level approach based on a quasilikelihood function and provides the population-averaged estimates of the parameters when accounting for correlated measurements (198). In order to confirm the significance of RNFL loss associated with OSA and obtain study subjects-specific estimates an additional multivariable model was constructed using the linear mixed-effects method which explicitly accounts for the correlations between paired eyes of subjects by adding a random effect (random intercepts and coefficients of subject and eye) in addition to the fixed effects (the examined predictors of interest). The model provides the conditional mean of the outcome given covariates and random effects.

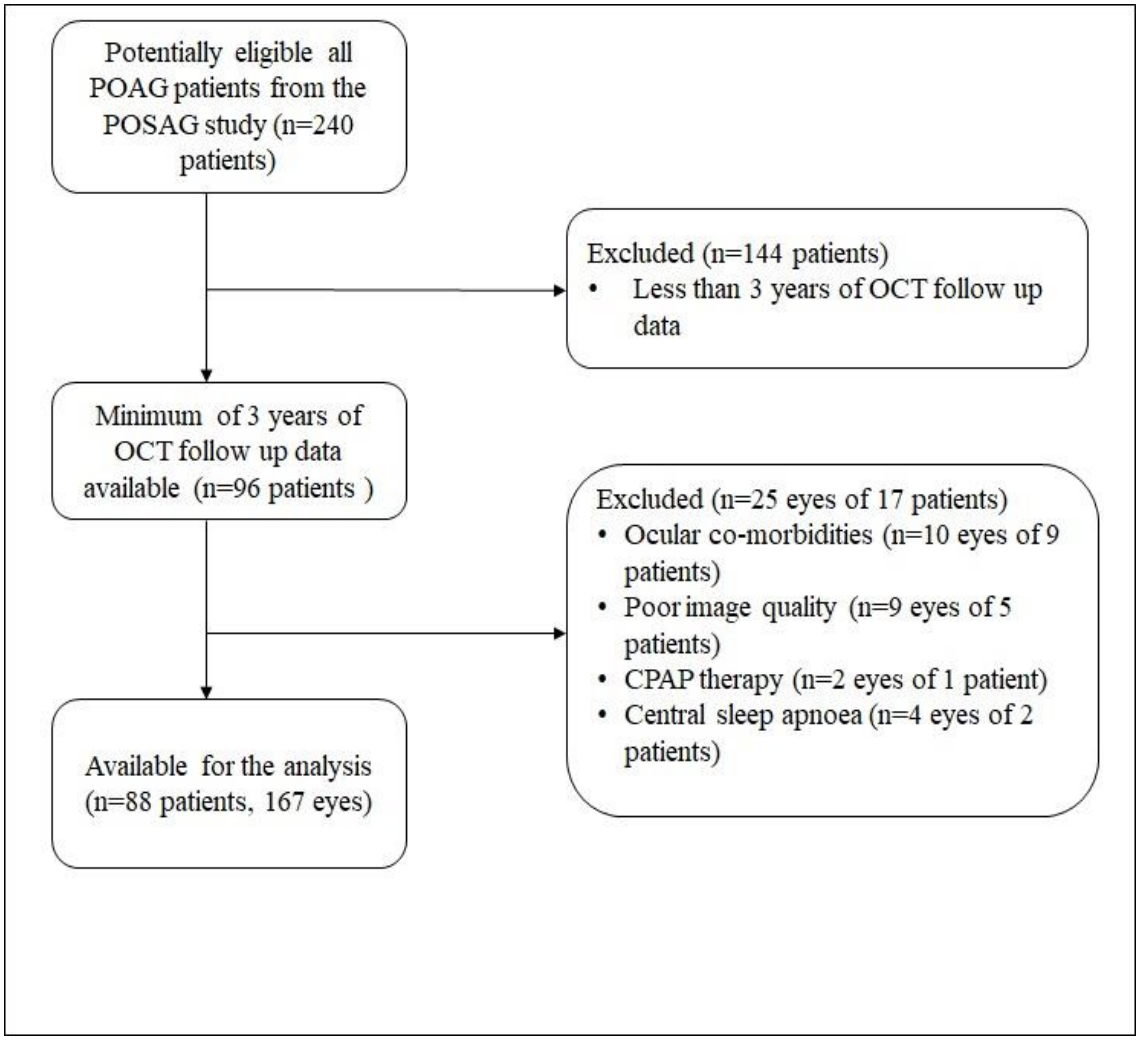
In addition, the analysis of variance (mixed model ANOVA) was used to compare RNFL thinning between OSA severity categories.

## 4.3 Results

A total of 167 eyes of 88 POAG patients were included in the analysis (Figure 4-2). Obstructive sleep apnoea was diagnosed in 54.5% of them and was moderate to severe in 21.6% of all patients. The median time separating the first and the last RNFL measurements was 4 (IQR-2.0) years and 66% of patients included in the analyses were diagnosed with POAG and established on hypotensive treatment before the first OCT scan used in this analysis was obtained. For between-group comparisons of baseline characteristics and the distribution of follow up periods in relation to the diagnostic sleep test please see Table 4-1 and Figure 4-3, respectively.

In keeping with the results reported in Chapter 3 for the cross-sectional associations between OSA and RNFL thickness, the AHI or the OSA status were not significant predictors of RNFL measured during the study visit (p=0.59 and p=0.081, respectively). However, average global RNFL thickness loss was significantly greater in patients with OSA than those without OSA (-1.1 ±1.5 µm/year versus -0.6 ±1 µm/year, respectively, p=0.013). The largest differences in RNFL thinning between the groups were observed in the superior and inferior quadrants (Table 4-2). On average, the greatest longitudinal changes in RNFL thickness were noted in people with severe OSA but the OSA severity categories and AHI were not significantly correlated with global RNFL thinning (Table 4-3 and Table 4-4). In a multivariable model adjusted for age, IOP, baseline RNFL thickness, corneal hysteresis, previous trabeculectomy, and a composite variable of cardiovascular co-morbidities, OSA status was independently associated with global RNFL progression (see Table 4-3 for the GEE model and Appendix 13 for the linear mixed effects model). The other significant predictors of RNFL loss in both models were: higher baseline RNFL thickness and cardiovascular co-morbidities. Higher IOP measured at the study visit was significantly associated with greater RNFL decline only in the linear mixed effects model. The peak IOP was a redundant co-variant in both models not associated with RNFL thinning (Beta= -0.01, p=0.48, GEE univariate model) and due to its correlation with the IOP measured at the study visit was removed from the adjusted models. The proportions of NTG patients among people with and without OSA were not different (Table 4-1) and there was no statistically significant interaction between OSA status (OSA vs No OSA) and the two POAG subgroups (NTG vs HTG) in relation to the change in RNFL thickness (GEE model; OSA vs No OSA*NTG vs HTG, p=0.63) which indicates that OSA does not preferentially affect structural progression in NTG patients (also see Table 4-5).

Figure 4-2. Study Flow Chart




Abbreviations: POAG= primary open-angle glaucoma, OCT=ocular coherence tomography, CPAP=continuous positive airway pressure.

Table 4-1. Baseline characteristics of participants included in the RNFL loss analysis.

| | No OSA | OSA | P value |
|---|---|---|---|
| Patients, n (%) | 40 (45.5) | 48 (54.5) | |
| Eyes, n (%) | 75 (44.9) | 92 (55.1) | |
| *Demographics* | | | |
| Male Sex, n (%) | 16 (40) | 29 (60) | 0.06 |
| Age (yrs), sd | 66.7±9.6 | 70.1±8.5 | 0.08 |
| BMI (kg/m2), sd | 25.9±3.5 | 28±4.4 | 0.02 |
| *OSA indices* | | | |
| AHI (per h), IQR | 2.25±2.2 | 11.8±12.8 | 0.000 |
| Mean SpO2 (%), sd | 93.2±1.5 | 92.3±1.3 | 0.008 |
| T90% (%), IQR | 4±50 | 45±105.8 | 0.002 |
| *Ocular indices* | | | |
| $MD_{study\ visit}$ (dB), IQR | -3.5±5.7 | -4.0±6.8 | 0.099 |
| #RNFL thickness baseline (µm), sd | 73±16.5 | 69.8±19 | 0.25 |
| RNFL thickness study visit (µm), sd | 70.9±18.4 | 66.1±17.1 | 0.08 |
| $IOP_{current}$ (mmHg), sd | 14.3±4.4 | 14.7±4.8 | 0.68 |
| $IOP_{supine}$ (mmHg), sd | 16.5±3.16 | 16.9±3.4 | 0.57 |
| $IOP_{peak}$ (mmHg), sd | 22.4±5.1 | 24±5.3 | 0.13 |
| CCT, (µm), sd | 547±40.6 | 547.6±29.9 | 0.56 |
| SER (D), sd | -0.98±3.2 | -0.97±4.3 | 0.99 |
| High myopia, n (%) | 8 (14.8) | 12 (15.4) | 0.93 |
| Hysteresis (mmHg), sd | 9.1±2.3 | 8.9±2 | 0.6 |
| Filtration surgery, n(%) | 20 (25) | 23 (24) | 0.96 |
| *Other data* | | | |
| Duration of OCT follow up, years, (sd) | 4.7 ±1.4 | 4.3±1.2 | 0.18 |
| NTG, n (%) | 15 (37.5) | 12 (25.5) | 0.25 |
| *Cardiovascular co-morbidities, n(%) | 21 (43.8) | 27 (56.3) | 0.56 |

Abbreviations: RNFL=retinal nerve fibre laye, AHI=apnoea-hypopnoea index, T90=time spent with oxygen saturation less than 90%, MeanSpO2=mean oxygen saturation, $IOP_{current}$=intraocular pressure recorded at the study visit using Goldmann tonometer, $IOP_{supine}$=intraocular pressure measured with IcarePro tonometer in a supine position (after 15 min lying down) at the study visit, $IOP_{peak}$=highest ever recorded intraocular pressure, CCT=central corneal thickness, NTG=normal tension glaucoma (POAG patients who never had IOP recorded above 21mmHg in either eye), *Cardiovascular co-morbidities =at least one of the following: diabetes, hypertension, ischemic heart disease, atrial fibrillation, cerebro-vascular accident, heart failure, over 20 pack year smoking history, #Global RNFL thickness measured on the first scan included in the progression analysis, SER= Spherical Equivalent Refraction, High myopia; SER≤ -4.0 D.

Figure 4-3. OCT follow up time in relation to the diagnostic sleep test in individual patients.

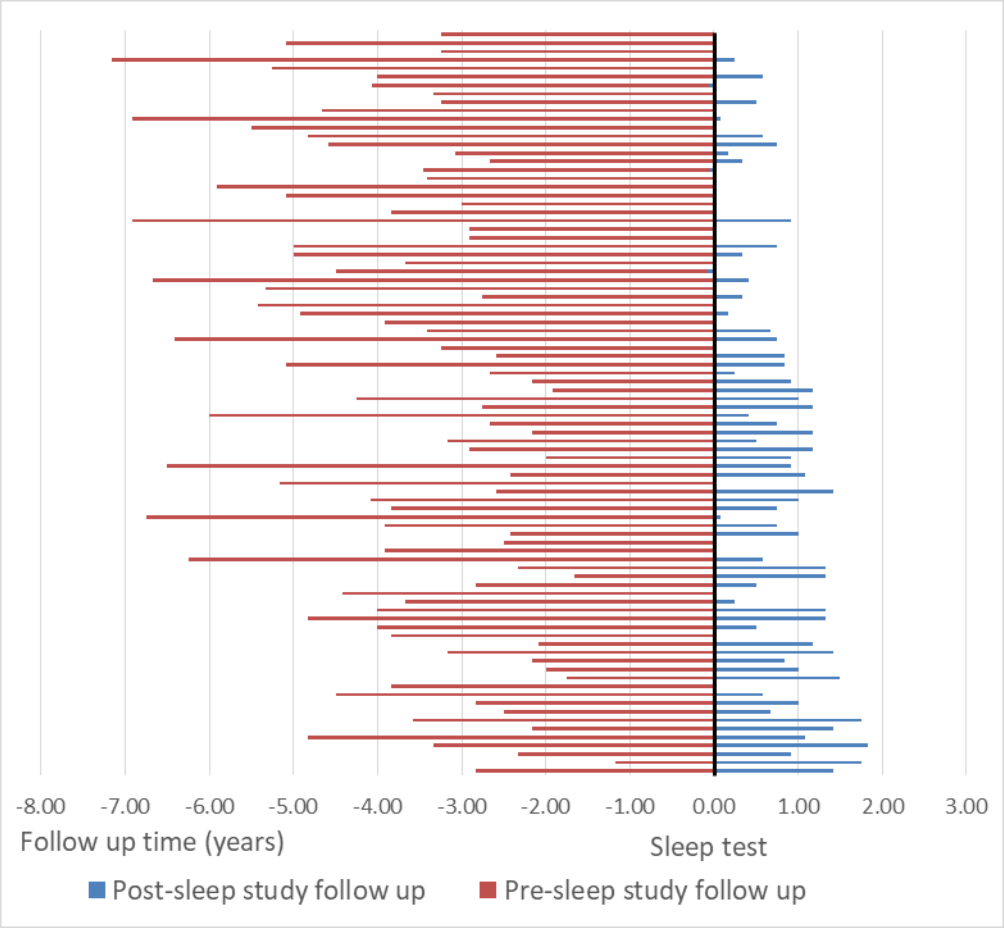



Table 4-2. RNFL thickness loss (μm/year) in OSA vs non-OSA group (GEE model).

| | No OSA | | OSA | | P value |
|---|---|---|---|---|---|
| | Mean±SD | 95% CI | Mean±SD | 95% CI | |
| Average Global RNFL | -0.6±1 | -0.85 to -0.36 | -1.12±1.5 | -1.4 to -0.8 | 0.013 |
| Superior quadrant | -0.59±2.1 | -1.06 to -0.11 | -1.28±2.1 | -1.71 to -0.85 | 0.034 |
| Nasal quadrant | -0.4±1.6 | -0.75 to -0.05 | -0.6±2.3 | -1.06 to -0.13 | 0.5 |
| Inferior quadrant | -0.86±2.4 | -1.41 to -0.32 | -1.77±2.9 | -2.37 to -1.17 | 0.028 |
| Temporal quadrant | -0.58±2.5 | -1.16 to -0.01 | -0.87±1.8 | -1.25 to -0.5 | 0.4 |

Abbreviations: RNFL=retinal nerve fibre layer, OSA=obstructive sleep apnoea

Table 4-3. Summary of GEE regression models examining the association between OSA status and the global RNFL thinning [$RNFL_{last}$-$RNFL_{baseline}$, µm/year ], (n=167 eyes).

| Model | Beta | Standard Error | 95% CI | Wald Chi-Suare | P value |
|---|---|---|---|---|---|
| *Unadjusted:* | | | | | |
| OSA (reference to no OSA) | -0.51 | 0.21 | -0.91 to -0.11 | 6.2 | 0.013 |
| AHI | -0.5 | 0.28 | -1.0 to 0.04 | 3.2 | 0.072 |
| *Adjusted* | | | | | |
| OSA (reference to no OSA) | -0.62 | 0.21 | -1.0 to -0.21 | 8.7 | 0.003 |
| CVS co-morbidities (reference to no CVS co-morbidities) | -0.47 | 0.23 | -0.92 to -0.03 | 4.3 | 0.039 |
| Baseline RNFL thickness | -0.016 | 0.0063 | -0.03 to -0.003 | 6.3 | 0.012 |
| $IOP_{current}$ | -0.062 | 0.036 | -0.13 to 0.01 | 2.9 | 0.09 |
| Previous filtration surgery (reference to no surgery) | -0.39 | 0.34 | -1.1 to 0.27 | 1.4 | 0.245 |
| Corneal Hysteresis | 0.038 | 0.039 | -0.038 to 1.1 | 0.97 | 0.326 |
| Age | 0.01 | 0.012 | -0.013 to 0.03 | 0.74 | 0.39 |

Abbreviations: OSA= obstructive sleep apnoea, Baseline RNFL thickness measured on the first scan included in the analysis, AHI=apnoea-hypopnoea index, $IOP_{current}$=intraocular pressure recorded at the study visit, CVS=cardiovascular co-morbidities (at least one of the following: diabetes, hypertension, ischemic heart disease, atrial fibrillation, cerebro-vascular accident, heart failure, over 20 pack year smoking history).

Table 4-4. Average global RNFL loss (µm/year) according to OSA severity (mixed model ANOVA).

| | No OSA (n=70 eyes) | Mild OSA (n=52 eyes) | Moderate OSA (n=26 eyes) | Severe OSA (n=10 eyes) | P value |
|---|---|---|---|---|---|
| Global RNFL loss | -0.58±1 | -0.98±1.0 | -1.18±1.0 | -1.32±1.01 | 0.15 |

Abbreviations: RNFL=retinal nerve fiber layer, OSA=obstructive sleep apnoea.

Table 4-5. Average global RNFL loss (μm/year ) according to POAG subgroups and OSA status.

| | NTG | HTG |
|---|---|---|
| No OSA | -0.54±0.73 | -0.64±0.8 |
| OSA | -0.95±1.04 | -1.26±1.05 |

Abbreviations: NTG=normal tension glaucoma, HTG=high tension glaucoma OSA=obstructive sleep apnoea.

### 4.4 Discussion

This study, which is one of the first to examine the relationship between OSA and structural glaucoma progression, found that POAG patients with untreated OSA had almost twice greater RNFL thickness loss than those without OSA. Moreover, OSA status as a binary predictor was independent of other ocular and systemic factors previously associated with RNFL thinning. The lack of statistically significant correlation between AHI and the longitudinal RNFL change could be potentially explained by a small number of patients with severe OSA. Only 5 out of 88 participants were diagnosed with severe OSA and the median AHI among all OSA patients was relatively low at 11.8. Yet, there was a clear difference in RNFL progression between the groups which was not explained by other factors. It is possible that with a larger representation of patients with severe OSA a dose response effect would have been demonstrated.

It has been proposed that OSA may be more relevant to glaucomatous neuropathy in patients with NTG rather than HTG (199). In fact, some studies exclusively examined the role of OSA in patients with NTG and excluded those with HTG (131,200,201). The current study found no differential effect of OSA on RNFL loss in the two POAG subgroups. This, in addition to the similar prevalence of OSA in NTG and HTG patients found in a larger sample of patients who participated in the study reported in Chapter 3, indicates that distinguishing these two subgroups is not necessary when the impact of OSA on glaucoma is examined.

It should be noted that most patients in this study had been already diagnosed with POAG and started on treatment before the first RNFL measurements used in the analysis were acquired. It is therefore not surprising that the peak IOP, which for most patients was the IOP recorded at the point of diagnosis, was no longer a predictor of RNFL progression. It was, however, a significant predictor of RNFL thickness in the previous study (Chapter 3, Table 2) which indicates that the neural damage in the cross-sectional analysis was largely a function of historically raised IOP. Notably, the average IOP readings obtained just prior to the sleep test in the current study showed an effective

control of IOP. At that point RNFL loss may have been less IOP dependent which could have helped to detect the impact of untreated OSA.

To my knowledge only one previous recently published study examined structural glaucoma progression in relation to OSA (202). In a retrospective design, the authors reviewed their PSG database and identified 25 POAG patients and 7 glaucoma suspects who had at least 3 years of follow up with OCT imaging. They performed an event-based analysis and found that the proportion of patients who progressed was significantly higher among those with moderate to severe OSA than in a combined no OSA/mild OSA group. Patients with severe OSA had 8.4-fold higher risk of RNFL progression although because of the small number of patients the confidence interval was wide (CI: 1.5-48.8). Furthermore, the analysis was not adjusted for any ocular co-variates, the rate of progression was not stated and a third of all patients were established on CPAP therapy or had undergone a surgery for OSA.

The current study has the advantage of a larger sample size, better defined RNFL monitoring period in relation to the OSA diagnosis, accounting for other ocular factors and including only patients with untreated OSA which eliminated the potential confounding effect of CPAP. The results are however in agreement with the previous study.

What is less certain is whether the differences in the magnitude of RNFL loss observed in the current study will translate into a future more severe functional visual impairment in OSA patients. This is not easy to predict as the correlation between longitudinal changes on OCT images and perimetry is generally poor with many studies reporting a discordance between progression defined by functional and structural outcomes (177,203). However, in general, faster rates of RNFL loss are associated with a higher risk of developing subsequent visual field defects. For instance, in a study by Yu et al. eyes with established POAG which progressed based on RNFL trend analysis had 8.4-fold increased risk of developing visual filed progression (204). In terms of the reported average rates of RNFL thinning in relation to developing functional visual loss a prospective longitudinal multicentre study by Diniz-Filho et al. is one of the largest of its kind (205). The authors examined 547 eyes of 339 patients with treated POAG and glaucoma suspects who were followed up for average of 3.9 years and had repeated RNFL measurements with the same OCT device as the one used in the current study. They reported that eyes which showed progression on standard automated perimetry had an average rate of RNFL loss of -1.02 µm/year which compared to -0.61 µm/year ($p=0.002$) in non-progressing eyes. These progression rates are close to those reported in the current study which would suggest that the RNFL thinning observed in the OSA group may be clinically significant. Further perspective on this provides a comparison of the average RNFL decline among OSA subjects with age-related RNFL thickness loss

in people with healthy eyes which in longitudinal studies was consistently reported at -0.5 µm/year (206,207). Thus, even before accounting for the differences in baseline average RNFL thickness which is much higher in people with non-glaucomatous eyes (100 µm in the study by Leung et al. vs 71 µm in the current study) and which is also an important negative predictor of RNFL loss, the estimated rate of structural progression in OSA patients was more than twice as fast as expected from aging alone.

This study exclusively examined subjects with POAG and it could be concluded that OSA is a risk factor for progression of glaucomatous damage. Indeed, the greatest change in RNFL thickness and the largest differences between patients with and without OSA were noted in the superior and inferior quadrants which are preferentially affected in POAG (208–210). However, this topographic pattern is not a unique feature of POAG. It has also been clearly described in Alzheimer's disease and multiple system atrophy where axonal loss predominantly affects the superior and inferior quadrants with relative sparing of the temporal and nasal regions (179). Therefore, it is also possible that the observed RNFL thinning is not specific to glaucomatous neuropathy but represents more general neurodegenerative process accelerated by chronic intermittent hypoxia. This hypothesis requires further examination in longitudinal studies of OSA patients without ocular diseases.

The main limitation of this study is its retrospective design. The RNFL data were extracted from scans acquired as part of routine practice which lacks the scrutiny of systematic approach to progression monitoring which can be implemented in a prospective design. I accounted for IOP readings obtained during the POSAG study visit and for the highest IOP readings documented in patients' records but not for average or repeated IOP measurements during the RNFL monitoring period which potentially could have also influenced the structural progression.

The RNFL loss was simply calculated based on the first and the last available OCT scans. A similar approach was adopted in some other retrospective studies which for example measured the rate of visual field loss in the context of cardio-vascular diseases (211). The limitation of this approach is a potentially higher measurement variability of the calculated progression rate compared to a trend-based analysis which uses a regression slope to estimate the rate of change in the examined parameter. The latter method is less sensitive to a sudden change and the variability among consecutive tests which are neutralised by the overall rate of change (212). This method however increases the risk of ignoring true progression events when changes occur in early stages of monitoring and particularly when the observation period is relatively short. Moreover, one of the main assumptions of the trend-based analysis is that the progression is a linear process which may not be true for all individuals, particularly those with more advanced glaucomatous damage when the floor effect is likely to limit the

ability of structural measurements to detect the true progression rate. However, the main disadvantage of trend-based analysis is the requirement for a large number of tests before the analysis can be considered reliable. In the current study a substantial proportion of patients had only one or less adequate quality OCT scans per year. Therefore, adopting a trend-based analysis would lead to further exclusions and a lower number of participants. This method is however more appropriate for prospective studies where more frequent measurements taken at regular intervals can be organised.

As already discussed, the relatively small number of patients with severe OSA may have affected the ability to detect statistically significant relationship between OSA severity metrics and RNFL loss as well as the precision around the estimated size of the longitudinal RNFL change in the severe OSA group. In addition, due to the fact that just over a third of the original pool of POAG patients met the eligibility criteria for this analysis, I cannot exclude a selection bias.

### 4.5 Conclusions

This study indicates that OSA may be an independent risk factor for progressive structural damage of the optic nerve in patients with POAG. However, the findings should be confirmed in a larger prospective study, ideally with concomitant rigorous monitoring for visual field progression. At present it is unclear whether the magnitude of structural damage which can be attributed to OSA is large enough to cause noticeable visual loss in peoples' lifetime.

It is possible that RNFL thickness could also be a useful surrogate marker in examining the role of OSA in the development and progression of other neurodegenerative disorders. For this, however, the impact of OSA on longitudinal RNFL changes should be first assessed also in people with healthy eyes.

# Chapter 5: CPAP related intraocular pressure increase at night in people with and without glaucoma (PAIR II study).

## Null hypothesis:

CPAP therapy in people with OSA does not change IOP at night.

### 5.1 Introduction

Obstructive sleep apnoea is commonly treated with CPAP applied by a nasal or a full face mask which maintains patency of the upper airway during sleep. CPAP is the first line treatment for moderate to severe OSA and, among currently used non-invasive treatment modalities, it is also the most effective one in controlling intermittent airway obstructions in sleep. At present, people with OSA and co-existing glaucoma receive standard treatment with CPAP; glaucoma status is not taken into account during the CPAP set up and titration process. However, whilst the long-term impact of CPAP on glaucoma is unknown, two previous studies showed that CPAP increases IOP at night (138,139). This is concerning as it means that CPAP could compromise the effect of IOP lowering therapies at night when IOP is already at its highest due to the recumbent sleep position and circadian variations. The influence of CPAP therapy on nocturnal IOP requires confirmation in further studies using newer tools which allow for accurate IOP monitoring whilst at the same time minimising sleep disruption. Crucially, we are yet to understand how IOP responds to CPAP in people with glaucoma in whom IOP control is of particular interest. With the exception of one patient with NTG, the two previous studies did not include glaucoma patients and IOP may plausibly behave differently in response to CPAP in people with glaucoma. A few scenarios are possible: i. CPAP has the same effect on IOP in glaucoma patients as in normal subjects, ii. IOP increases substantially more in people with glaucoma who are generally more prone to IOP fluctuations in response to a range of challenges to its regulation, iii. IOP lowering treatment is protective and mitigates CPAP related IOP increase.

Ultimately, only a prospective interventional randomised trial can confidently assess the impact of CPAP on important glaucoma outcomes, both in terms of its safety as well as potentially beneficial effects in slowing glaucoma progression. In the development of such studies which, given the slowly progressive nature of the optic nerve damage, will inevitably require long treatment and monitoring periods, it is important to consider the immediate effects of CPAP, their magnitude and potential mechanisms. This knowledge could help us to understand whether and how positive airway pressure therapy could be

optimised to prevent or minimise undesirable IOP increases. Any rise in IOP might negate the potential beneficial effect of controlling OSA on the eye. Such knowledge could inform the design of future clinical studies but also, importantly, guide the current practice of treating OSA patients with co-morbid glaucoma.

This Chapter will focus on examining the impact of CPAP on nocturnal IOP in people with OSA with and without POAG.

## 5.2 Methods

### *5.2.1 Study Design*

This is a prospective observational controlled study designed to assess nocturnal IOP in people with POAG and control subjects at baseline and during nocturnal CPAP therapy 4-6 weeks from its initiation.

The study was approved by the East of England – Cambridge South Research Ethics Committee (REC number 16/EE/0074, Appendix 14) on 30th March 2016. The tenets of the Declaration of Helsinki were followed and written informed consent was obtained from all participants. The first subject was recruited on 24th March 2017 and the last patient completed the study on 10th February 2018.

### *5.2.2 Participants and settings*

All invited participants were newly diagnosed with OSA and had clinical indications for CPAP therapy. POAG patients were established on IOP lowering therapies and had adequately controlled IOP. This was confirmed during POSAG visits or, for patients who did not take part in the POSAG study, during the screening visit. Any modalities of IOP lowering therapies, including ocular hypotensive drugs, previous laser treatment or trabeculectomy were allowed. As POAG is very rare among people younger than 40 years old, and to avoid age discrepancy between the groups, only potential subjects aged 40 and above were invited to join the study. Further matching of participants in the two groups for BMI and OSA severity was contemplated. However, after conducting a feasibility assessment it was concluded that due to the complex nature of the study which required multiple visits, the anticipated low number of potentially eligible participants with POAG and with expected recruitment rate of 50%, the study would fail to reach the desired sample size within one year of recruitment if precise prospective matching for these variables was attempted. Nevertheless, in an attempt to mitigate any imbalances between the groups the majority of POAG patients had been enrolled first and the control subjects who had a BMI within +/- 5kg/m$^2$ range of already recruited individuals with POAG and the same OSA severity category (mild, moderate, severe), were invited subsequently. Furthermore, as the main IOP measurements were to be taken during

sleep at fixed intervals for all patients, people with insomnia, short sleep duration, irregular sleep habits, delayed or advanced sleep phase were excluded.

*Summary of eligibility criteria*

*Inclusion criteria:*

All participants

i. Newly diagnosed, untreated OSA with indications for CPAP
ii. Age ≥ 40 years
iii. Able to give informed consent and attend for the study visits

Glaucoma patients

i. Treated primary open-angle glaucoma

*Exclusion criteria:*

All participants

i. Known or suspected pregnancy
ii. Any contraindications to rebound tonometry, including: corneal scarring, microphtalmos, buphthalmos, nystagmus, keratoconus, abnormal central corneal thickness, corneal ectasia, active corneal infection, and corneal dystrophies.
iii. Concomitant eye diseases known to affect IOP, including: treated wet age related macular degeneration (ARMD), central retinal vein occlusion (CRVO), branch retinal vein occlusion (BRVO), uveitis and diabetic retinopathy.
iv. Indications to start CPAP treatment urgently unless the study visits could be organised at short notice to avoid a delay in treatment initiation.
v. Acute infectious diseases precluding hospital admission.
vi. Irregular sleep pattern and insomnia.

Control group

i. Any form of glaucoma
ii. Inability to undergo screening ophthalmic examination

There were two recruitment sources:

i. People with and without POAG who had completed the POSAG study (described in Chapter 2) who had been found to have OSA associated with relevant symptoms were subsequently referred for assessment in the Sleep Disturbance Clinic (SDC) at the Royal Papworth Hospital where decisions on further management were made. Following review in SDC, those who met the above eligibility criteria were invited to take part in the

study and provided with a Participant Information Sheet (PIS). After a minimum of 3 days they were approached by telephone to discuss the study and enquire about their interest to participate. Those who chose to contribute were invited to attend for the study visit (Visit 1).

ii. Patients with and without glaucoma who attend the SDC during the study recruitment period and were recommended CPAP treatment for newly diagnosed OSA were screened for eligibility and, if appropriate, were approached according to the procedure described above. Those who agreed to take part in the study were invited to attend for the screening visit (Visit 0).

*5.2.3 Study Plan*

Depending on the recruitment pathway, participants attended for 2 or 3 study visits. The study protocol illustrating these pathways and study procedures at each visit are depicted in Figure 5-1.

Screening Visit

Only participants recruited from the SDC who required a full eye examination and/or respiratory rPG attended for this visit which took place in the Glaucoma Clinic at Hinchingbrooke hospital. All patients diagnosed with OSA based on nocturnal oximetry had to undergo rPG to confirm the nature of SDB and assess its severity more precisely. Those, who already had rPG or a PSG as part of their diagnostic work up, were not required to repeat it. Participants recruited from the POSAG study had undergone rPG already.

Consented participants underwent a full eye examination which allowed confirmation of POAG in participants treated in other centres, exclusion of eye diseases which could potentially affect IOP readings in control subjects and enabled me to obtain up to date ocular measurements. The following tests were carried out:

1. Automated visual fields (HFA, SITA-Fast, Carl Zeiss Meditec, Jena, Germany)
2. Slit-lamp biomicroscopy and gonioscopy (Haag-Streit International, Koeniz, Switzerland)
3. Goldmann applanation tonometry (Haag-Streit International, Koeniz, Switzerland)
4. Central corneal thickness (Pachmate 2, DGH Technology Inc., Exton, USA)
5. Corneal hysteresis (Ocular Response Analyser, Reichert, N.Y., USA )

6. Fundus photography
7. OCT of the retina (Heidelberg Engineering Inc. MA, USA).

rPG sleep studies were conducted according to the same procedure as described in Chapter 3.
Participants who met the eligibility criteria following the screening visit were invited for Visit1.

All participants were asked to regularise their sleep habits, particularly 7 days prior to Visit 1 and 2, to optimise alignment in their sleep-wake pattern between the visits and facilitate nocturnal IOP measurements at planned intervals. POAG patients continued taking their eye drops as recommended by their ophthalmologists and were specifically advised that they must not alter the time, frequency or doses of their glaucoma medications between Visit 1 and Visit 2. If any changes in IOP lowering therapies were needed during the study period, patients were advised to contact the study team to discuss their ongoing participation.

Visit 1

Participants attended the RSSC in the evening (7-8pm) and were placed in a dedicated quiet ward separate from other patients. This area is used predominantly as a day ward and so has overnight availability. Up to four patients were admitted at a time. Once participants had given informed consent, the following assessments and measurements were carried out:
Baseline assessment: A brief medical history focused on cardio-respiratory co-morbidities, glaucoma management and medications was taken. Demographic and anthropomorphic variables, including: age, weight, height, BMI and neck circumference were collected. Bedside spirometry was performed.
Repeated measurements: IOP was measured in each eye using a rebound tonometer (IcarePro, Finland Oy) which was described in Chapter 2. Six single IOP measurements were obtained each time and a mean IOP was recorded as displayed by the device. If the variation between the six measurements was outside of acceptable limits set by the manufacturer, which is indicated by a yellow or red light, the measurements were repeated. Blood pressure (BP) was checked at the same time as IOP using a non-invasive automated sphygmomanometer. Ocular perfusion pressure (OPP) was calculated based on IOP, systolic (SBP) and diastolic (DBP) blood pressure according to the following formula: OPP=2/3 [DBP+1/3(SBP-DBP)]-IOP(138). Oxygen saturation (SpO2) and heart rate (HR) were monitored overnight continuously using a finger probe pulse-oximeter.

The first measurements were performed between 9 and 10pm in the upright position and repeated 30 minutes later in the supine position. After this, participants were advised to switch the lights off when they felt ready with the expectation that the majority would fall asleep between 10 and 12pm. They were allowed to sleep in their habitual position but the number of pillows used and the angle of the head end of the bed was noted for consistency between visit 1 and visit 2.

All the above measurements were repeated every 2 hours starting from 10pm and finishing at 8am. Participants were gently woken up and instructed to remain lying down in bed. The measurements were taken with the participants supine under dim light to minimise sleep disruption. Two experienced operators were taking measurements at a time for each patient. Blood pressure was taken first and was immediately followed by IOP measurements. Unless IOP readings required repeating, both tests were completed in less than one minute. Sleep position prior to awakening the subject was recorded. After the final IOP measurements in the morning, participants underwent OCT-A imaging which will be described separately in Chapter 7.

The final part of Visit 1 was training the participants to self-measure IOP using another rebound tonometer (iCareHome, Finland, Oy) previously described in Chapter 2. Participants took the device home and were instructed to record IOP in the daytime every 2 hours between 10am and 8pm on two separate days: before they started CPAP and 4-6 weeks into the treatment. They were also provided with an eye lubricant to apply at home in order to reduce any risk of corneal abrasion from repeated IOP measurements.

Once all research procedures of Visit 1 were concluded, patients met with a CPAP Practitioner who initiated treatment with an auto-titrating CPAP (S9 Escape, ResMed). This is currently a standard practice in our centre. For participants' convenience, this clinic appointment was co-ordinated with Visit 1. Patients were instructed how to use CPAP, fitted with an appropriate mask and had an opportunity to practice with the treatment. They then followed the usual treatment pathway which involves another review by a CPAP Practitioner 7 to 10 days later when the auto-CPAP is changed to a fixed pressure device based on the 90$^{th}$ percentile pressure level recorded by the auto-CPAP machine.

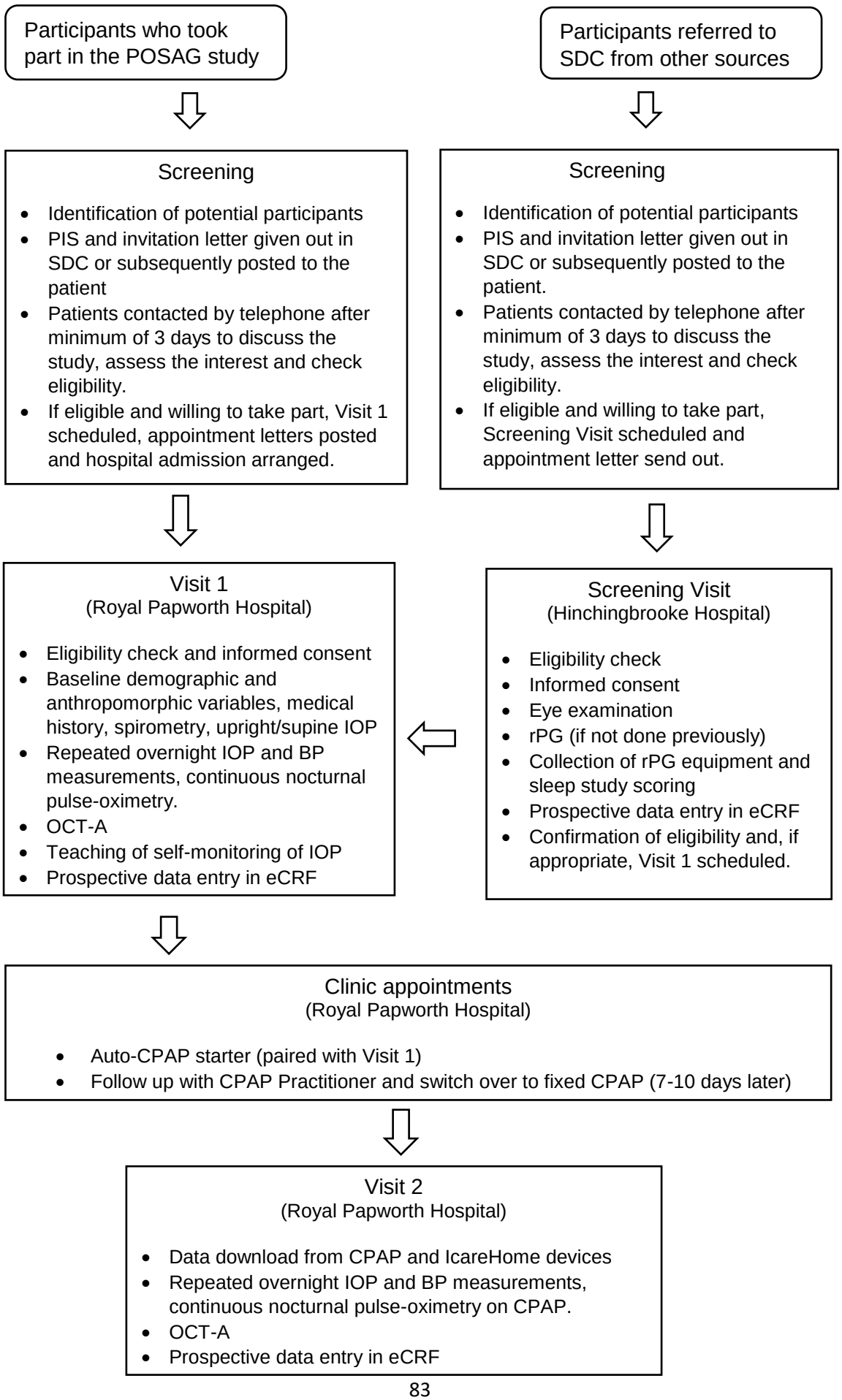
Visit 2

Participants returned for the second overnight assessment 4-6 weeks after starting CPAP. The hours of machine usage and the pressure level set on the device were recorded. The IcareHome tonometers were collected and the IOP data recorded by the participants were downloaded.

All the measurements, following the same procedure as for Visit 1, were repeated this time with participants using CPAP. They were housed on the same ward and awakened at exactly the same time intervals starting from 10pm and finishing at 8am.

OCT-A imaging were repeated on the following morning straight after participants took the CPAP off. This concluded Visit 2.

Figure 5-1. Study flow diagram



### *5.2.4 Study outcomes*

Primary:

Change in IOP measured repeatedly at night in both eyes between Visit 1 (off CPAP) and Visit 2 (on CPAP).

Secondary:

i. Differences in IOP change (ΔIOP; $IOP_{CPAP}$ -$IOP_{baseline}$) between the groups.
ii. Change in nocturnal OPP, BP, SpO2 between the visits and differences in responses to CPAP between the groups.
iii. Change in diurnal IOP self-measured at home before and after CPAP treatment.
iv. Correlation of IOP change (ΔIOP) in response to CPAP with: OSA severity (AHI), CPAP level, BMI, %predicted FVC.
v. Change in OCT-A indices before and after CPAP treatment (this outcome is reported separately in Chapter 7).

### *5.2.5 Statistical considerations*

#### *5.2.5.1 Sample size*

The only published data which allowed sample size estimation for the purpose of this study came from paper by Pepin et al (139). The authors of this study measured IOP at baseline and one month into CPAP treatment in 12 subjects without glaucoma. They found that nocturnal IOP increased significantly from 14.8 (0.8) to 18.3 (1.2) mmHg (mean+/-SEM). Based on these data, and assuming 0.5 within subjects correlation between the two sessions, 11 participants per group were required to detect this difference separately in each group with 80% power and 5% significance (two-tailed t-test for the difference between two dependent means, G*Power; version 3.0.10, Appendix 15). Allowing for 25% dropouts between the visits, the current study aimed to recruit 15 participants per group.

It should however also be noted that the study design allows for comparisons of multiple correlated measurements taken at 5 time points over the two visits. For this reason, in analysis of the primary outcome F rather than T statistic was used. It is therefore likely that the above calculated power would have been achieved even with smaller sample size. However, no sufficient data to feed the F test power calculation model was available at the point of designing this study, thus it was thought that the above calculation was the closest estimation possible.

*5.2.5.2. Statistical Analysis*

Continuous data are presented as mean (SD) or median (IQR) depending on the distribution. Categorical data are reported as percentages. Normality was checked using Shapiro-Wilk test and by assessment of skewness and its standard error. Comparisons of baseline characteristics between the groups was performed using independent-samples T-test or, if the data were not normally or approximately normally distributed, and in case of categorical variables, using the Mann-Whitney U test. Summary indices, such as ODI and mean SpO2, were compared between Visit 1 and Visit 2 using paired-sample T-test or non-parametric Wilcoxon test, as appropriate.

A two way mixed-model ANOVA with Visit 1 and Visit 2, IOP measurement time points ( 00am, 2.00am, 4.00am, 6.00am, 8.00am ) and eye (right and left) as within-subject factors, and the study groups (POAG vs Control) as between-subject factors was built to assess nocturnal IOP responses to CPAP. In addition, for between group comparisons of IOP change between the two visits, ΔIOP defined as: $IOP_{Visit2}$-$IOP_{Visit1}$, was calculated for each monitoring time point and each eye and plotted as a within-subject factor in a mixed-model ANOVA. This approach allowed for any possible differences in baseline IOP between the groups to be taken into account. Post hoc pairwise comparisons with Bonferroni correction were performed for any variables with a significant within-subject effect and interactions. Sphericity was evaluated and in cases where sphericity was violated, the sphericity-corrected P value is reported.

Due to a substantial proportion of missing data, the ANOVA model could not be used for comparisons of pre- and post-treatment diurnal IOP values obtained at home by the participants. Here, mean IOP values were compared separately for each measurement time point with a paired-sample T-test and also compared as a pre- and post-treatment mean of these means.

To examine predictors of CPAP related IOP change, linear univariate regression models with ΔIOP ($IOP_{Visit2}$-$IOP_{Visit1}$) as a dependent variable and CPAP level, AHI and BMI as independent variables were constructed. Pearson's correlation coefficients were also calculated. The level of significance for each comparison was set at $p < 0.05$. All analyses were conducted using SPSS software version 22.0 (IBM SPSS, IL, USA).

## 5.3 Results

### *5.3.1 Included Participants*

A total of 28 participants, including 15 POAG patients and 13 control subjects were recruited. Of these, one POAG patient and one control participant were unable to tolerate CPAP and were excluded from the analysis. The POAG patient who could not use CPAP because of claustrophobia returned for Visit 2 and had repeated overnight measurements taken without wearing CPAP which allowed for a limited assessment of between visits IOP reproducibility (Appendix 16). The control subject reported difficulties sleeping with the CPAP mask despite trying several different interfaces. She withdrew from the study after Visit 1. Of the 26 participants who successfully completed all study visits, 14 (11 POAG patients and 3 control subjects) were diagnosed with OSA via the POSAG study route and 12 (3 POAG patients and 9 controls) were routine referrals to SDC from other sources. Baseline characteristics of participants are outlined in Tables 5-1 and 5-2. All but one of the participants were diagnosed with moderate to severe OSA. One control subject had mild OSA but had previously failed other treatment modalities and had clinical indications for a trial of CPAP. Control subjects had significantly higher ESS scores. They were also slightly younger and required higher CPAP levels to control their OSA, although the differences between the groups in these variables did not reach statistical significance, possibly as a result of a small sample size. POAG patients had adequately controlled IOP as indicated by average IOP readings which were taken with a Goldmann tonometer during POSAG/Screening visit and with IcarePro at Visit 1. They had elevated IOP at the point of diagnosis and mild visual field impairment.

Table 5-1. Baseline characteristics of participants who successfully completed PAIR II study and CPAP related data (T-test or Mann-Whitney U test).

| | POAG N=14 | Controls N=12 | P value |
|---|---|---|---|
| Age (yr), sd | 68.5±8.2 | 61.6±10.6 | 0.072 |
| Sex (% Male) | 57.1 | 75.0 | 0.35 |
| BMI (kg/m2), sd | 32.8±5.2 | 35.2±6.2 | 0.71 |
| Neck (cm), sd | 41.5±6.2 | 43.8±5.4 | 0.34 |
| FEV1 predicted (%), IQR | 97.5±22 | 100.5±38 | 0.70 |
| FVC predicted (%), sd | 96.3±15.7 | 103±21 | 0.35 |
| FEV1/FVC, sd | 0.81±0.05 | 0.8±0.08 | 0.90 |
| $SBP_{upright}$ (mmHg), sd | 140.1±16.4 | 141.8±22.4 | 0.82 |
| $DBP_{upright}$(mmHg), sd | 82.3±11.2 | 84.7±6.2 | 0.52 |
| AHI (per h), IQR | 32.5±25.5 | 31.8±34.8 | 0.64 |
| ESS score (baseline),sd | 9.1±4.7 | 13.3±5 | 0.041 |
| CPAP level (cmH2O), sd | 10.8±2.9 | 13.0±2.9 | 0.074 |
| CPAP usage (h/night),sd | 5.1±1.7 | 5.3±1.6 | 0.99 |
| Hypertension,% | 35.7 | 66.7 | 0.123 |
| Antihypertensive medications (%) | 28.6 | 58.3 | 0.133 |
| Type 2 DM | 14.3 | 16.7 | 1 |
| Hyperlipidaemia | 35.7 | 41.7 | 0.76 |
| IHD | 7.1 | 8.3 | 0.91 |
| CVA | 7.1 | 0 | 0.78 |
| AF | 0 | 8.3 | 0.74 |

Abbreviations: BMI=body mass index, FEV1=forced expiratory volume in one second, FVC=forced vital capacity, SBP=systolic blood pressure, DBP=diastolic blood pressure, AHI=apnoea-hypopnoea index, ESS=Epworth Sleepiness Scale, CPAP=continuous positive airway pressure, DM=diabetes mellitus, IHD=ischaemic heart disease, CVA=cerebro-vascular accident, AF=atrial fibrillation, POAG=primary open-angle glaucoma.

Table 5-2. Ocular characteristics of participants with POAG (N=14).

| | Right Eye | Left Eye |
|---|---|---|
| MD (dB), sd | -2.2±2.3 | -2.5±1.9 |
| Cup/Disc ratio, IQR | 0.75±0.2 | 0.7±0.3 |
| Average RNFL thickness (µm),IQR | 77±14.5 | 75.8±22 |
| CCT (µm), IQR | 551.5±48 | 547±46 |
| Corneal Hysteresis (mmHg), sd | 9.8±3.5 | 8.4±1.5 |
| IOP at the point of POAG diagnosis, (mmHg), sd | 24.3±6.4 | 22.3±4.2 |
| IOP (mmHg) Goldmann at POSAG/screening visit, sd | 15.9±3.5 | 16.5±3.5 |
| IOP (mmHg) upright, IcarePro, Visit 1, sd | 15.1±2.6 | 15.6±2.7 |
| IOP lowering treatment: | | |
| Prostaglandin analogue (%) | 86 | 79 |
| Beta blocker (%) | 21 | 29 |
| Carbonic anhydrase inhibitor (%) | 29 | 29 |
| Alpha agonist (%) | 7 | 7 |
| Combination of ≥ 2 topical hypotensives (%) | 36 | 36 |
| Previous SLT (%) | 14 | 14 |

Abbreviations: MD=mean deviation, RNFL=retinal nerve fibre layer, CCT=central corneal thickness, IOP=intraocular pressure, POAG=primary open-angle glaucoma, SLT=selective laser trabeculoplasty.

*5.3.2 Response to CPAP*

Adherence to CPAP was adequate with average machine usage between the visits of over 5 hrs/night in each group (Table 5-1). CPAP treatment resulted in a significant reduction of nocturnal oxygen desaturations and improvement in subjective daytime sleepiness in both groups (Table 5-3).

Table 5-3. Changes in nocturnal oximetry and ESS score with CPAP treatment (paired-sample T-test).

| | Visit 1 | Visit 2 | P value |
|---|---|---|---|
| POAG group (N=14) | | | |
| ODI (per h), sd | 15.3±8.4 | 7.7±6.6 | 0.012 |
| Mean SpO2 (%), sd | 94.1±0.89 | 96±1.2 | 0.000 |
| ESS score, sd | 9.14±.4.7 | 5.8±3.2 | 0.001 |
| Control group (N=12) | | | |
| ODI (per h), sd | 21.7±21 | 7.4±9.7 | 0.006 |
| Mean SpO2 (%), sd | 94±1.9 | 95.9±1.3 | 0.003 |
| ESS score, sd | 13.3±5 | 8±5.6 | 0.002 |

Abbreviations: ODI=oxygen desaturation index, SpO2=oxygen saturation, ESS=Epworth Sleepiness Scale, POAG=primary open-angle glaucoma.

*5.3.3 Primary Outcome: CPAP related changes in nocturnal IOP*

IOP measured with subjects using CPAP (visit 2; 5 repeated measurements in both eyes at: 00am, 2am, 4am, 6am, 8am) was significantly higher than at baseline (visit1; the same time intervals as per visit 2) with a mean increase of 2.6±2.2 mmHg across both groups and all measurement time points (F=36, p=0.000) (Figure 5-2). This was also true when data were analysed separately in each group (POAG group; mean IOP increase: 2.2±2.6 mmHg, F=10.1, p=0.007, Control Group; mean IOP increase: 3.1±1.7 mmHg, F=40, p=0.000). The difference between the groups in IOP change was not statistically significant (mean difference in ΔIOP: 0.99±4.4mmHg, p=0.27) (Figure 5-3). It is worth noting that IOP measurements taken at 10pm in supine position off CPAP at both study visits were not different indicating reproducibility of IOP measurements between the visits (Visit 1 $IOP_{10pm}$:17.6±2.8, Visit 2 $IOP_{10pm}$:17.1±3.0, F=1, p=0.33). At visit 2, with participants being asleep and wearing CPAP for just 1-2 hrs between 10pm and 00am, IOP has already increased by 2.6±2.7 mmHg (F=24.7, p=0.000) when the first measurements on CPAP were taken. There was no significant difference in the magnitude of IOP change across the 5 measurement time points (F=1.6, p=0.2). In 50% of participants IOP increased by ≥5mmHg at one or more time points. The highest IOP readings were recorded in two POAG patients at 8 am reaching 28mmHg and 31mmHg in the right eye, which represented 12.1mmHg and 13.2mmHg increase compared with the same time at Visit 1, respectively. Overall, changes in IOP between the visits were more variable in people with POAG than in control subjects. For instance, the only patient

who had a mean nocturnal IOP lower during CPAP therapy (-1.5 mmHg) and a patient with the highest mean IOP increase (+8.5 mmHg) were both in the POAG group (Figure 5-4).

Of the examined potential predictors of IOP change, only CPAP level was positively correlated with a mean increase in nocturnal IOP. In a regression model it could explain 19% of variability ($R^2$) in IOP change between the visits (Table 5-4 and Figure 5-5).

In a post-hoc analysis, the ANOVA model which examined the differences between the POAG group and the control group in nocturnal IOP change was adjusted for the CPAP level. This resulted in a decrease in the mean difference in IOP change between the groups and an increase in the p value (mean difference in ΔIOP; unadjusted model: 0.99±4.4mmHg, p=0.27 vs adjusted model: 0.35±4.4mmHg, p=0.7).

Missing data

There were no missing data for the primary outcome analyses. However, three POAG participants and two control subjects woke up and discontinued CPAP between 6 am and 8 am. Therefore, the 8 am IOP measurements were taken off CPAP in those patients. In four of them, the 8 am IOP readings were lower than those obtained at 6 am. Whilst they remained lying in bed for all IOP checks, it is possible that overall differences in nocturnal IOP between the visits would have been slightly larger if all 8 am measurements were taken on CPAP.

Figure 5-2. Nocturnal IOP before and during CPAP therapy. For between-visit comparisons only measurements taken between 00 am and 8 am were included.

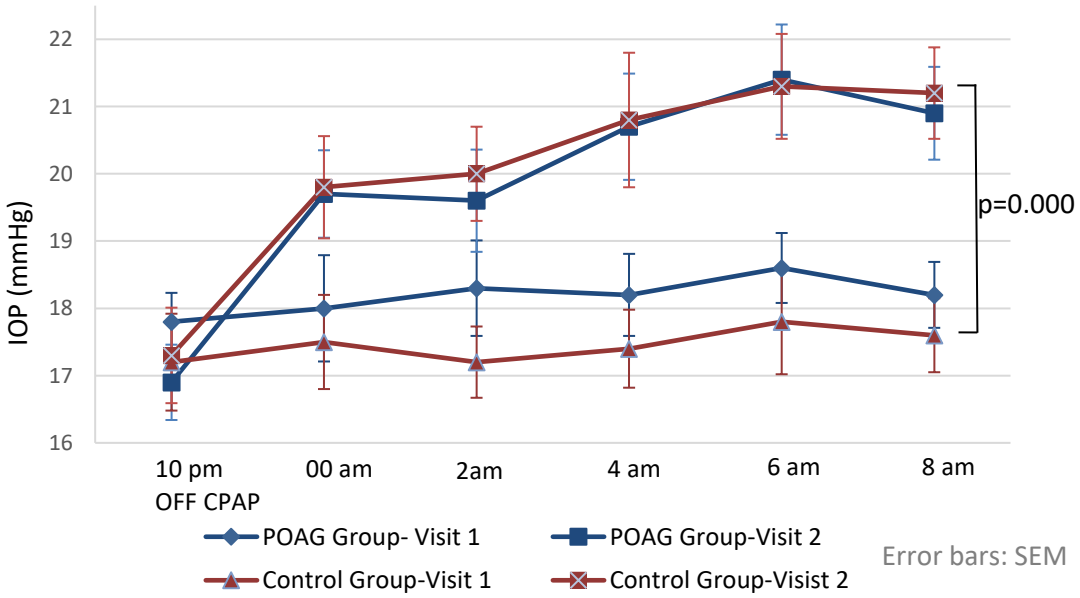


Figure 5-3. Comparison of CPAP related IOP change between the groups (mixed-model ANOVA with post-hoc Bonferroni transformation).

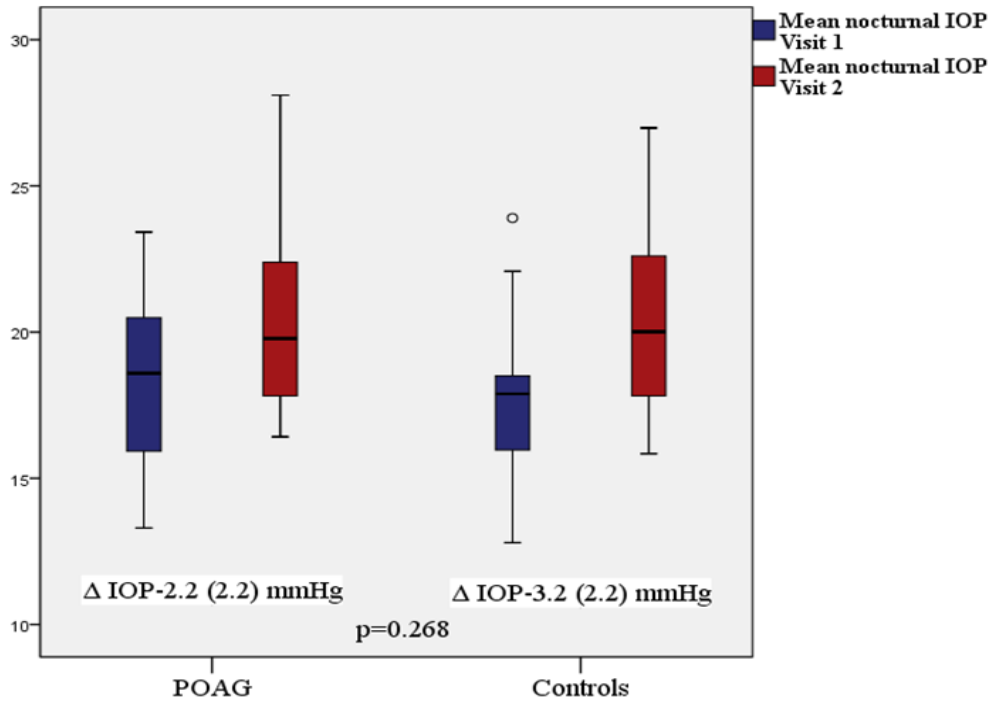
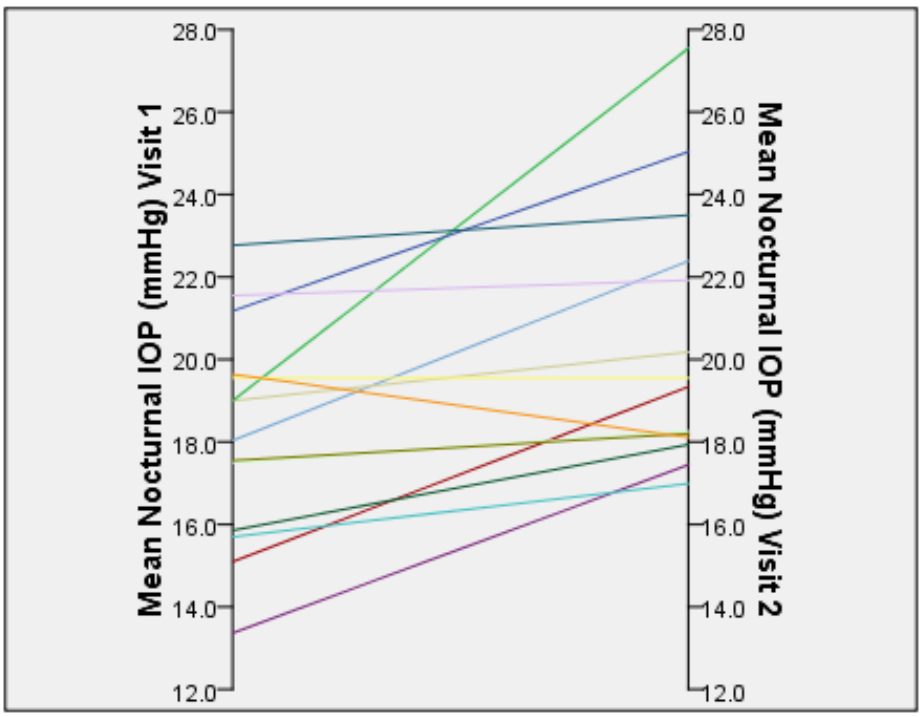


Figure 5-4. Changes in mean nocturnal IOP between Visit 1 and Visit 2 in each group. Each line represents a patient (mean of both eyes).

A. POAG Group




B. Control Group

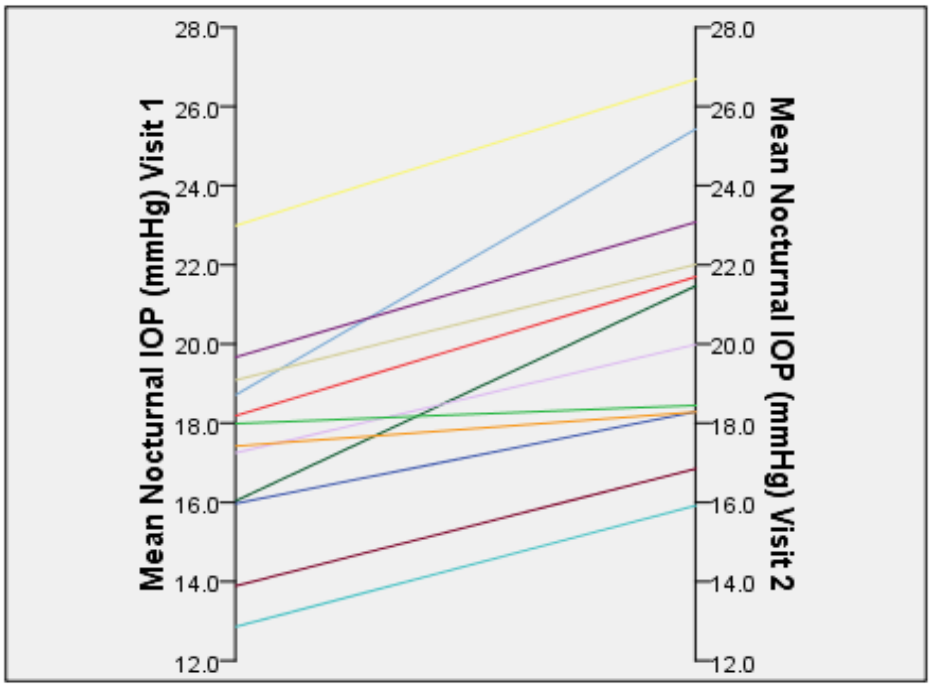
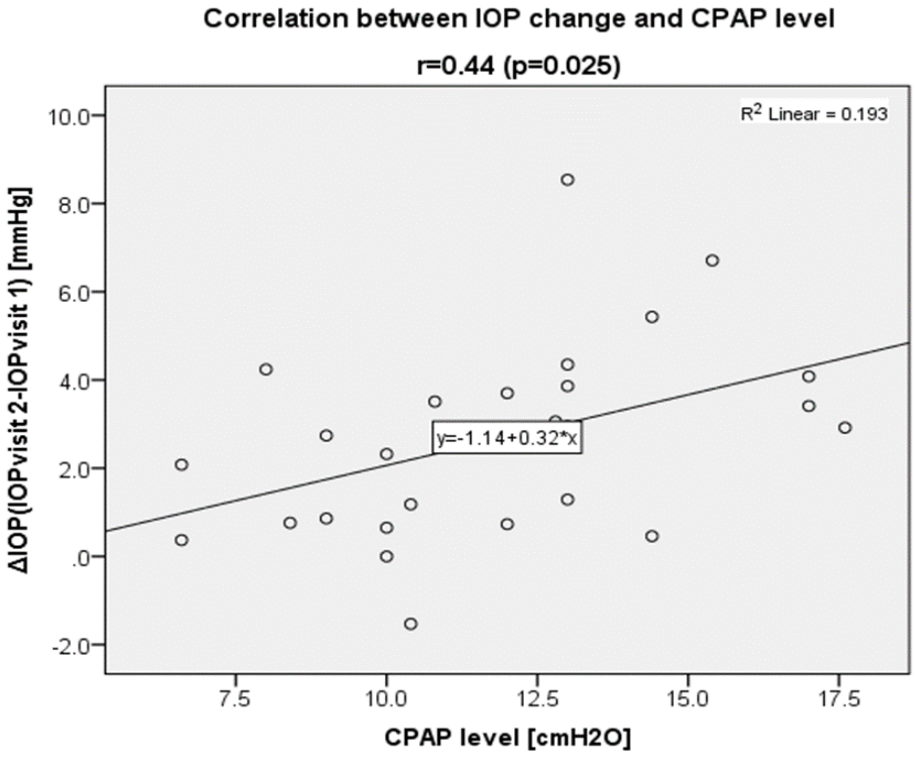


Table 5-4. Summary of the univariate linear regression models examining potential associations between selected variables and mean nocturnal IOP change between Visit 1 and Visit 2.

| Model | N | $R^2$ | Beta | t value | P value | 95% CI |
|---|---|---|---|---|---|---|
| CPAP level | 26 | 0.19 | 0.32 | 2.4 | 0.025 | 0.04 to 0.6 |
| AHI | 26 | 0.01 | -0.013 | -0.5 | 0.625 | -0.7 to 0.04 |
| BMI | 26 | 0.005 | 0.027 | 0.34 | 0.74 | -0.14 to 0.19 |
| FVC %predicted | 26 | 0.008 | 0.011 | 0.45 | 0.66 | -0.04 to 0.06 |

Abbreviations: CPAP=continuous positive airway pressure, AHI= apnoea-hypopnoea index, BMI=body mass index, FVC=forced vital capacity.

Figure 5-5. Scattered plot representing a correlation between CPAP level and mean nocturnal IOP change between Visit 1 and Visit 2.



*5.3.4 Secondary outcomes*

Changes in nocturnal BP and OPP

There was a small but significant reduction in OPP at Visit 2 when all subjects were entered into the mixed-model ANOVA (mean ΔOPP: -2.7±4.4 mmHg, F=9.5, p=0.005). However, there was also a significant interaction between the groups and the visits (F=8.2, p=0.009). Post-hoc analysis showed that OPP was significantly reduced only in the control group (POAG group; ΔOPP: -0.19±3.6mmHg, F=0.041, p=0.84, Control Group; ΔOPP: -5.1±5.2mmHg, F=11.8, p=0.006) (Figure 5-6).

Subsequent analyses indicated that the OPP changes were largely driven by IOP increase but there was also a contribution from BP changes. In the control group, there was a trend to reduction in SBP but in the POAG group SBP had increased (Control group; ΔSBP: -6.1±11.1 mmHg, F=3.5, p=0.087, POAG group; ΔSBP: 5±6.4 mmHg, F=5, p=0.038,) counterbalancing the effect of IOP elevation on calculated OPP.

DBP was not significantly different during CPAP therapy in control subjects but increased in POAG patients with a difference close to being statistically significant (Control group; ΔDBP: -1.4±5.5 mmHg, F=0.7, p=0.41, POAG group: ΔDBP: 2.6±4.9 mmHg, F=4.4, p=0.057) (Fig. 5-7 and 5-8).

Figure 5-6. CPAP related changes in calculated nocturnal OPP. *For between-visit comparisons only measurements taken between 00 am and 8 am were included.*

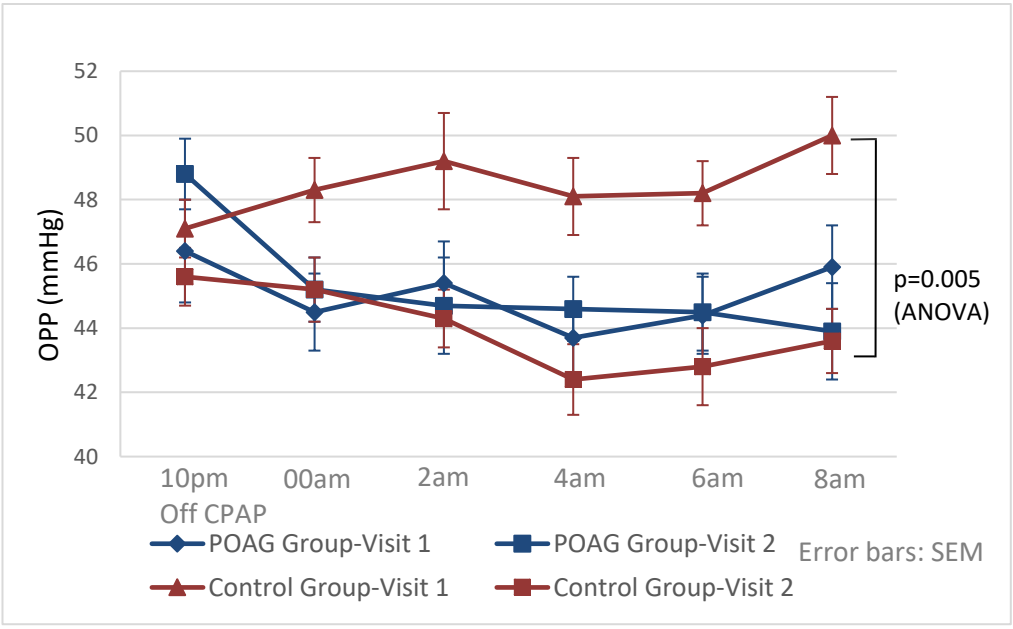


Figure 5-7. Changes in SBP between visits in each group. *For between-visit comparisons only measurements taken between 00 am and 8 am were included.*

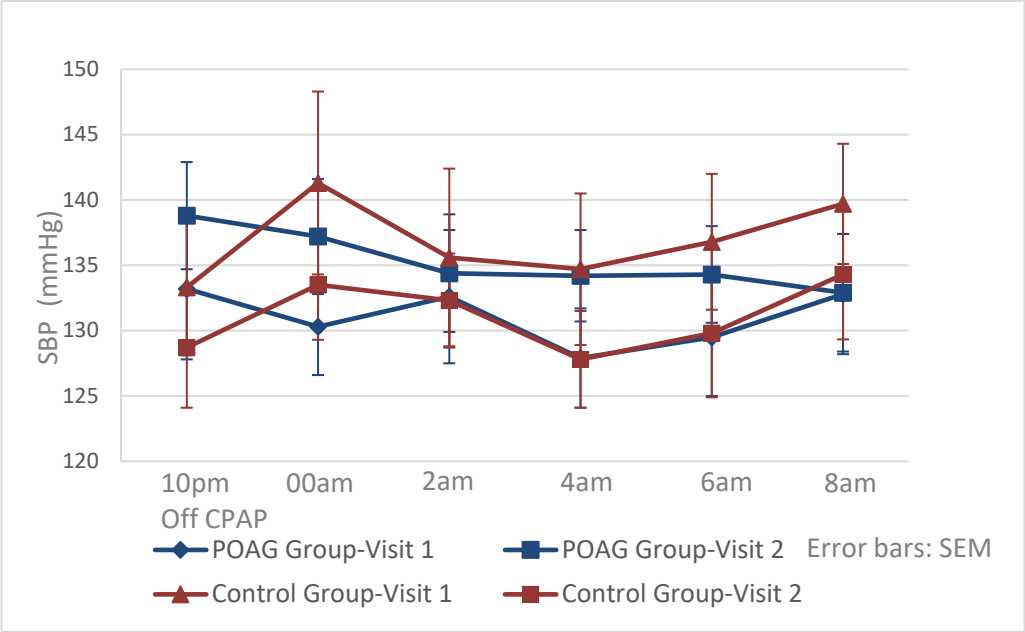
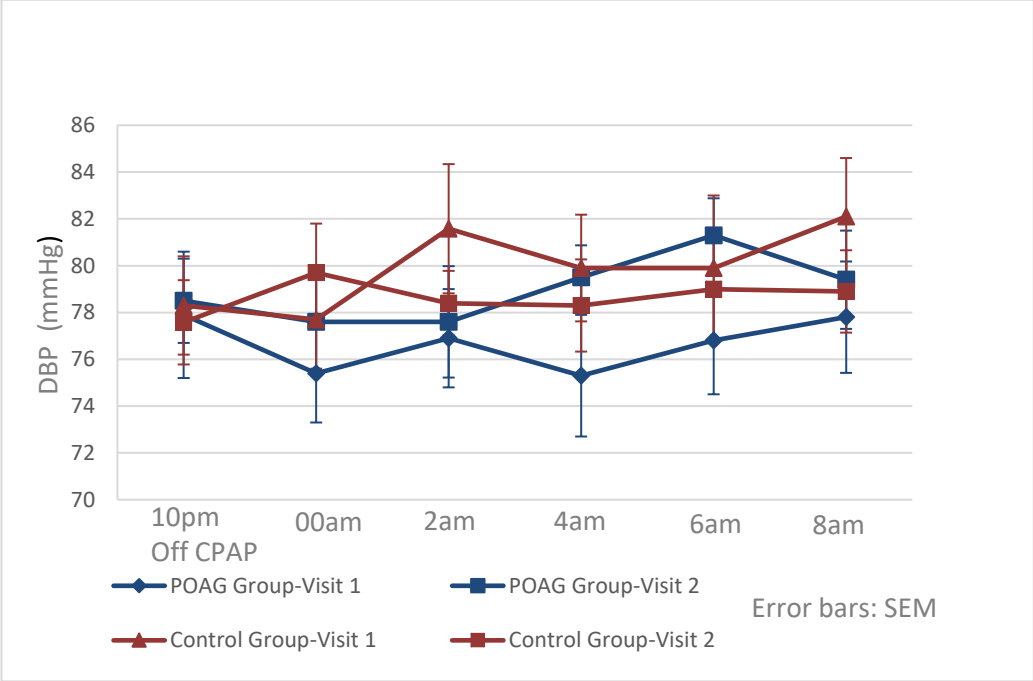



Figure 5-8. Changes in DBP between visits in each group. *For between-visit comparisons only measurements taken between 00 am and 8 am were included.*



Changes in diurnal IOP self-measured at home before and during CPAP treatment.

After a short training, 20 out of 26 participants were able to self-measure IOP. Unfortunately, there was a substantial proportion of missing data due to the following reasons: missed recordings at one or more time points, measurements taken outside of the scheduled time, IOP measured only in one eye or no valid IOP measurements obtained despite multiple attempts. Only 4 participants collected all of the 24 required measurements (two eyes, 6 time points throughout the day, pre- and post- CPAP treatment). Data produced by them were analysed using repeated measures ANOVA which showed no significant change in diurnal IOP after CPAP therapy (ΔIOP: 0.77±1.7mmHg, F=0.9, p=0.42). Due to a large proportion of missing data, imputation methods were deemed inappropriate. Instead, T-statistics was used to compare IOP readings at each time point and for each eye separately. This approach may have not increased the power but allowed data to be included from participants who provided reliable but incomplete measurements. In addition, pooled mean IOP recording pre- and post- CPAP were compared with a paired sample T-test. There was a good correlation in mean IOP measurements before and after 4-6 weeks of CPAP treatment but no significant change in daytime IOP was detected with any of these tests (Table 5-5 and 5-6).

Table 5-5. Comparison of pre- and post- CPAP diurnal IOP at all time points for both eyes (paired sample T-test).

| | Right Eye | | | | Left Eye | | | |
|---|---|---|---|---|---|---|---|---|
| Time | N | ΔIOP* | t value | P value | N | ΔIOP | t value | P value |
| 10.00 am | 13 | -2.8±4.8 | -2.1 | 0.06 | 11 | -0.9±4.5 | -0.68 | 0.51 |
| 12.00 am | 15 | -1±4 | -9.8 | 0.35 | 10 | -1.9±2.8 | -2.1 | 0.06 |
| 2.00 pm | 14 | 0.21±2.4 | 0.34 | 0.74 | 12 | -1.7±4.4 | -0.13 | 0.9 |
| 4.00 pm | 11 | 0.00±4.1 | 0.00 | 1 | 10 | -1.2±3.3 | -1.16 | 0.27 |
| 6.00 pm | 13 | -1.1±5.1 | -0.76 | 0.46 | 12 | -1.3±2.8 | -1.56 | 0.15 |
| 8.00 pm | 15 | 0.27±4.4 | 0.24 | 0.82 | 11 | -3.6±5.6 | -2.1 | 0.06 |
| Mean IOP | 20 | -3.2±2.3 | -0.61 | 0.55 | 19 | -1.2±3.0 | -1.8 | 0.1 |

* $\Delta IOP = IOP_{post\text{-}CPAP} - IOP_{pre\text{-}CPAP}$

Abbreviations: CPAP=continuous positive airway pressure, IOP-intraocular pressure

Table 5-6. Correlation of mean diurnal IOP pre- and post- CPAP for each eye (Pearson correlation test). *Please note that the p value relates to the correlation test rather than comparison of means.*

| Right Eye N=20 | | | | Left Eye N=19 | | | |
|---|---|---|---|---|---|---|---|
| $IOP_{pre\text{-}CPAP}$ | $IOP_{post\text{-}CPAP}$ | r | P value | $IOP_{pre\text{-}CPAP}$ | $IOP_{post\text{-}CPAP}$ | r | P value |
| 15.5±3.3 | 15.9±3.6 | 0.77 | 0.000 | 15.3±4 | 16.5±3.8 | 0.7 | 0.001 |

Abbreviations: CPAP=continuous positive airway pressure, IOP-intraocular pressure

## 5.4 Discussion

### *5.4.1 CPAP related IOP increase*

The main rationale for this study was to examine overnight IOP changes in response to CPAP therapy in people with POAG as no data existed for such patients. It was also important to verify findings of the two previous small studies which examined the impact of CPAP on nocturnal IOP in people without glaucoma but used different IOP measurement tools. Kiekens et al. monitored IOP every 2 hours over a 24-hour period at baseline and one month after starting CPAP (138). They used a Perkins tonometer which is a handheld version of the gold standard Goldmann tonometer and also requires the prior application of topical anaesthetic and fluorescein each time before measurements are taken. Therefore, this technique can cause potentially relevant sleep disruption and the measurements cannot be taken immediately after awakening the patient. As it relies on a skilful application of force required to maintain a uniform applanation of the surface of the cornea, it is operator dependent and thus there is a potential risk of bias in an unblinded design. In a sample of 21 patients, the authors of that study reported statistically significant change in IOP at 2, 4, 6 and 8 am. The average IOP increase was not reported in the paper but based on the published graph it was approximately 2.3 mmHg (Appendix 17). Daytime IOP was not different between the two sessions at any time point. In the second study by Pepin et al. IOP was measured every hour during the 24-hour period with an applanation tonometer (Tonopen XL) which also requires instillation of a local anaesthetic (139). This device is considered less accurate in measuring IOP when compared with Goldmann and other tonometers, and tends to give underestimated readings in the higher range of IOP (213–215). Using this tonometer, the authors found that in 12 patients who returned for repeated measurements on CPAP, nocturnal IOP increased by a mean of 3.5 mmHg.

In line with these two studies, in the current study IOP increased at night during CPAP therapy in the control subjects but I have now shown that this effect is also seen in people with POAG. The IOP change was slightly larger in the control group than in the POAG group (3.2 vs 2.2 mmHg) but this difference was not statistically significant and could be potentially explained by baseline imbalances between the groups. Specifically, the groups were not perfectly matched for CPAP level, with POAG patients receiving slightly lower CPAP. This may be relevant as in the regression analysis CPAP level was the only factor positively correlated with a mean IOP increase. It is also worth noting that individual IOP responses to CPAP were quite uniform in the control group but more variable in the POAG group. IOP variability defined as the change during periods longer than 24-hour and IOP fluctuation defined as the change within 24 hours are well documented in POAG and greater than in people with healthy eyes (216). In this context, variable individual responses to CPAP among POAG patients may reflect intrinsic features of IOP regulation in glaucoma.

Another possibility is that ocular hypotensive medications play a role in shaping individual changes in IOP during CPAP exposure. It could be speculated that eye drops have some protective effect but differ in their ability to mitigate CPAP induced IOP increase much as they differ in their efficacy in lowering nocturnal IOP. For instance, several studies demonstrated that prostaglandin analogues provide more consistent coverage for the nocturnal period than beta-blockers or anhydrase inhibitors (217–219). Brimonidine, a selective alpha2-adrenergic receptor agonist, effectively lowers diurnal IOP but has little effect during the nocturnal period (220). There is also evidence that adding a carbonic anhydrase inhibitor to a prostaglandin analogue monotherapy results in greater reduction of nighttime IOP than when a prostaglandin analogue is combined with a beta-blocker (221). Whether any of these medications are also more effective in controlling nocturnal IOP in the context of CPAP therapy is unknown. Complicating matters further is the fact that patients with OSA have heightened sympathetic activity at night so, potentially, beta-blockers may be more effective in this population but this has not been studied. In the current study, most patients were applying eye drops at night and were established on prostaglandin analogues but there was a range of hypotensive agents used in different combinations and due to relatively small sample size the effect of individual therapies could not be elucidated.

There were no significant changes in daytime IOP self-measured before and after CPAP treatment. Due to missing measurements and a different IOP monitoring strategy these data are less robust than for the nighttime period. Nevertheless, the results are consistent with the findings of the two previous studies, neither of which found significant changes in diurnal IOP. Importantly, Kiekens et al. demonstrated that IOP normalised

just 30 min after CPAP discontinuation. This indicates that the impact of CPAP on IOP is largely limited to the time that it is worn, which is predominantly during the sleep period.

*5.4.2 Potential mechanisms of nocturnal IOP elevation*

Several hypotheses have been proposed to explain why CPAP causes IOP elevation. Pepin et al. argued that normal nyctohemeral IOP rhythm is lost in OSA and that CPAP acts primarily by restoring this rhythm. Using the cosinor model for biological rhythms they categorised 18 patients into 3 subgroups: those who had a nocturnal rhythm (28%), a diurnal rhythm (22%) and those with no apparent rhythm (50%) at baseline (Appendix 18). Twelve of these patients returned for the second session when they had IOP measurements performed while using CPAP and they all exhibited a nocturnal rhythm then. Based on this, the authors concluded that any concerns about CPAP induced IOP changes should be disregarded. However, in light of available data, the question arises as to whether this unequivocal interpretation is justified and the hypothesis credible.

There is a large volume of evidence that IOP has a 24-hour rhythm with higher values at night and lower during the day. This is consistent across different age groups, people with healthy and glaucomatous eyes and has been demonstrated using several different IOP measurement techniques, including recently developed continuous non-invasive IOP estimation with a contact lens sensor (CLS) (222–225). The most important factor in nocturnal IOP elevation is the change in posture with IOP being higher in a supine than in a sitting position (226). There is also a circadian component to IOP regulation. Aqueous formation exhibits circadian rhythmicity; possibly as a result diurnal activity of the sympathetic nervous system its production is higher during the day and lower at night. However, as the nighttime aqueous outflow facility is reduced proportionally more, in most people, nocturnal IOP increases even when controlled for habitual body position (227,228). In their study, Pepin et al. measured IOP in a sitting position during daytime hours (from 8:00 am to 8:59 pm) and in a supine position at night (from 9:00 pm to 7:59 am). If, as is claimed, OSA reversed diurnal IOP rhythm in some patients, it would have to have a profound effect on circadian IOP regulation to overcome the postural change. To my knowledge this has not been previously reported for any other condition or in any population of patients.

In contrast with this study, the current study and also the study by Kiekens et al. indicate that the normal IOP rhythm is preserved in people with OSA. Although in the three studies IOP data were collected and analysed in slightly different ways certain comparisons are possible. In the study by Pepin et al. mean diurnal (sitting) IOP at baseline was 15.4 mmHg and nocturnal (supine) IOP was 14.8 mmHg which is consistent with the reported loss of nycthemeral rhythm in the majority of participants in that study. In the current study, mean diurnal (sitting) IOP was 15.4 mmHg but nocturnal

(supine) IOP increased to 17.9mmHg in all participants. In the study by Kiekens et al. mean daytime (supine) IOP was 16.4 mmHg but during the sleep period (supine) it increased to 17.5 mmHg. Furthermore, the authors reported that the mean of all trough-peak differences in IOP was 6.7 mm Hg at baseline and 9.0 mmHg during CPAP therapy. In the current study, nocturnal IOP increased between the visits by ≥5mmHg at one or more time points in 50% of participants. In some, this difference was more than 10mmHg in the early morning hours. In the study by Pepin et al. nighttime IOP appeared higher when measured on CPAP across all three subgroups, including in patients who were deemed to have nyctohemeral IOP rhythm preserved at baseline (Appendix 18). Therefore, in all three studies these net increases in IOP seem to be in addition and beyond what could be expected from 24-hour IOP rhythm restoration.

Contemplating further the data published by Pepin et al. a few other observations are noteworthy. The authors determined individual IOP rhythms based on measurements performed during one 24-hour session. One danger of this approach is that even short-term intra-subject IOP measurement reproducibility, particularly in terms of acrophase and bathyphase is limited. Mean diurnal and nocturnal IOP and the IOP amplitude measures are more consistent. (226,229–231). Pepin et al. classified IOP rhythms as a diurnal (ie, between 8:00 AM and 8:59 PM) or nocturnal based on the time of the acrophase. Whether one off acrophase measurements truly represented one's IOP rhythm would have to be determined by repeated measurements. In addition, in benchmark studies which investigated circadian IOP rhythms nocturnal period was limited to a dark i.e. sleep period (Appendix 19). Here the authors chose to extend it to 11 hrs. However, as the participants were allowed to sleep in their habitual hours and the average sleep time was only 5.7 hours, for almost half of the nocturnal period the measurements were not performed in sleep. Also, the follow up time between the baseline measurements and the measurements performed on CPAP varied widely between 1 month and 6.7 months. To what extent all these factors may have influenced rhythm classification and assessment of the subsequent response to CPAP is unclear. It should also be noted that adherence to CPAP was low, averaging only 3.6 hours. Three out of 12 patients who returned for repeated measurements were not using CPAP at all at home. Yet, it was reported that one night of CPAP use in the sleep laboratory restored their IOP rhythm which was either inverted or absent at baseline. This is surprising and would rather point to more immediate mechanisms than IOP rhythm restoration. For CPAP to alter biological processes, such as blood pressure control, endothelial dysfunction, sympathetic activation at least several weeks of regular use with coverage of the whole sleep period is required (109,232).

It has also been argued that CPAP may be raising IOP by restoring slow wave sleep the proportion of which is substantially reduced in severe OSA. Slow wave sleep was thought

to be associated with highest IOP in a couple of early studies in which subjects undergoing polysomnography were awakened for repeated IOP measurements (233,234). In the study by Pepin et al., with CPAP treatment the proportion of slow wave sleep increased from 6% to 13%. This still gives little opportunity to, by chance, capture slow wave sleep during scheduled one hourly measurements so that it would make a difference to the mean nocturnal IOP. Nonetheless, in a more recent study where IOP was measured continuously with CLS on non-OSA subjects without the need to awaken participants, the findings of the previous studies were disputed (235). In contrast, the IOP signal values were significantly higher during rapid eye movement (REM) sleep and progressively decreased during the non-REM stages reaching the lowest points in slow wave sleep.

From the physiological perspective a fall in IOP in non-REM sleep makes more sense as it is well established that sympathetic activation is higher in REM than in slow wave sleep (236). Circulating catecholamines could contribute to IOP increase by augmenting aqueous humour production through the stimulation of the ocular β-adrenergic receptors. It is well documented that interventions which reduce sympathetic stimulation of the ciliary epithelium, such as administration of topical or systemic beta-blockers, cause a reduction of IOP. OSA enhances sympathetic activity in all sleep stages. CPAP can restore autonomic balance at night and this effect carries over to wakefulness so that, for instance, blood pressure is reduced both at night and during the day (237–239). Pepin et al. proposed that one of the reasons why CPAP normalises nyctohemeral IOP pattern could be by the reduction of diurnal sympathetic tone. Whilst this is possible, it is unclear why CPAP would then have the opposite effect on nocturnal IOP. If augmented sympathetic activity was an important factor in IOP regulation it would be expected that patients with OSA would have higher baseline IOP both at night and during the day and that it would be reduced during both periods by CPAP therapy.

All these considerations should be taken into account when trying to understand the physiological mechanisms responsible for IOP change. The IOP rhythm restoration hypothesis per se offers little insight into these mechanisms. In an attempt to pursue it further, an assumption that OSA somehow either phase shifts the circadian clock or influences mediators of circadian rhythm which are also relevant to IOP regulation would have to be made. By causing sleep fragmentation OSA certainly has the potential to dysregulate circadian rhythms of various biological processes. So far, there is no convincing evidence that it affects the rhythmicity of the master circadian clock. For instance, most studies have not shown an altered melatonin profile in OSA subjects or that CPAP therapy changes melatonin secretion (240–242). Similarly, studies which looked at the profiles of various hormones including cortisol, which could be potentially important to IOP entrainment, did not find consistent evidence that these are altered in

OSA (243–245). Nevertheless, circadian dysregulation is still a relatively underinvestigated area in the OSA field and much more is to be learnt, particularly when it comes to clock gene expression.

As for the above hypothesis, based on the presented arguments I would conclude that whilst certain aspects of it, such as establishing whether circadian regulation of IOP is truly altered in OSA, are worth further exploration the current evidence does not support the notion that CPAP acts by simply restoring nyctohemeral IOP rhythm.

Kiekens et al. and others favoured the pressure transmission hypothesis according to which positive pressure generated by the CPAP machine is passed through the intrathoracic space to the ocular venous system impairing aqueous humour outflow and thereby increasing IOP. In the current study, there was a positive association between CPAP level and the magnitude of IOP increase which gives some support to this hypothesis. However, only 19% of the variance in IOP change could be attributed to CPAP level indicating that other mechanisms may be more important. Furthermore, while the average CPAP level in the current study was 12cmH2O, the participants of the study by Kiekens et al. received surprisingly low pressures which averaged at only 6cmH2O, yet the nocturnal IOP did increase. Further examination of this hypothesis and the association between CPAP level and IOP will be conducted in Chapter 6.

What may also matter is the control of repeated intermittent upper airway obstructions of OSA which may be causing rapid nocturnal IOP fluctuations. Respiratory effort against the occluded upper airway during apnoeic events generates large negative intrathoracic pressure swings which can reach values lower than -50 cmH2O, compared to levels of approximately -10 cmH2O in quite, unobstructed breathing. This in turn can increase right-sided venous return by enhancing the pressure gradient from extrathoracic to intrathoracic veins which would facilitate aqueous outflow and momentarily lower IOP. In a well-designed study Lundmark et al. demonstrated an immediate decline in IOP during the Mueller manoeuvre. This manoeuvre simulates the acute haemodynamic effects of negative intrathoracic pressure during an obstructive apnoea (246). Healthy volunteers were first trained to breath in against an occluded mouthpiece connected to a manometer and to reproducibly generate two levels of negative pressure: -20cmH2O and -40cmH2O. IOP was measured at baseline, 5-15s into the manoeuvre and immediately upon recovery using a pneumatic tonograph. There was a significant fall in IOP during the manoeuvre. Further, the authors showed a dose-response relation between the inspiratory effort and the magnitude of IOP change. Interestingly, oesophageal pressure measurements performed during sleep in OSA patients indicate that respiratory effort during upper airway occlusion is lower in REM than in non-REM sleep (247). This could

be another potential explanation for why IOP may be lower during slow wave sleep than in REM sleep.

The previously mentioned CLS device recently developed for continuous 24-hour measurements of IOP is a promising tool and may also give some insight into the pattern of IOP changes in OSA. The device consists of a silicone contact lens with embedded strain gauges which capture circumferential changes at the corneoscleral junction (248). The assumption behind this technology is that variations in IOP lead to changes in ocular volume and dimensions which are being detected by the micro-sensor. The device output is expressed in mVeq and cannot be easily converted to mmHg. Therefore, it provides data only on relative changes in IOP rather than absolute values and its ability to estimate IOP in comparison to other methods is yet to be established. Nevertheless, it does show some promise in certain situations such as when profiling IOP change at night.

So far only one small study has been published in people with OSA using the CLS device (122). In that study, IOP fluctuations were recorded overnight in 7 patients with moderate to severe OSA who were undergoing polysomnography. The focus was to compare CLS output during apnoeic events with this during non-obstructed breathing. The sampling frequency was every 5 minutes with the measurement time of 30 seconds. For this reason, only apnoeas longer than 20 seconds were selected for the analysis. The authors found that IOP recorded during apnoeic events was significantly lower than during normal breathing. However, it was also noted that the amplitude of IOP signal fluctuations related to changes in breathing pattern was 11 times smaller than the difference between minimum and maximum values on the overall nocturnal curve which, of note, was in keeping with normal nyctohemeral IOP increase. Whether these micro-fluctuations of CLS signal during apnoeic and non-apnoeic breathing truly represent IOP changes in keeping with those demonstrated during Mueller manoeuvre is yet to be established.

The tonometer used in the current study allowed us to measure IOP probably closer to transition from sleep to wakefulness than devices used in the two previous studies (138,139). Nevertheless, the measurements were still performed in wakefulness and it is unclear whether the potential impact of obstructed breathing on IOP could have been captured.

Lastly, one further mechanism potentially contributing to IOP increase during CPAP therapy is a change in the volume of extracellular body fluid. Nocturia and nocturnal polyuria are common complaints of patients with untreated OSA. They have been attributed to raised levels of atrial natriuretic peptide (ANP) generated in response to mechanical stretch of the atria during negative intrathoracic pressure swings. The main effect of ANP is reduction of extracellular fluid volume by increasing renal sodium

excretion and nighttime urine production (249). CPAP lowers plasma levels of ANP and by this mechanisms improves nocturia and reduces the volume of urine produced at night (250). This in turn leads to plasma volume expansion and lowering of haematocrit (251–253). A relative increase in extracellular fluid can contribute to IOP elevation. By analogy, the water drinking test which was first developed as a provocation test for glaucoma work up but later abandoned as a diagnostic tool due to its poor discriminative properties and low reproducibility, is still occasionally used to estimate peak IOP. Consumption of a relatively small volume of fluid such as 500ml can significantly increase IOP in people with glaucoma (254). Further, ANP itself may be involved in IOP regulation (255). ANP is present in aqueous humour and there is some evidence that it increases uveoscleral outflow (256,257). Intravenous injection of ANP or increasing the endogenous ANP within the physiological range lowers IOP (258). Thus, CPAP therapy by normalising augmented ANP secretion could increase IOP.

### *5.4.3 Clinical significance of CPAP related nocturnal IOP increase*

The recognition that IOP control throughout the 24-hour cycle is important in glaucoma management has been a significant paradigm shift in the last decade (259). It comes with challenges related to IOP monitoring outside office hours and the selection of treatment which would cover the 24-hour period.

One of the first issues to consider when discussing the potential long-term impact of a CPAP related nocturnal IOP increase on glaucoma progression is whether the magnitude of the demonstrated IOP change is large enough to be clinically relevant.

Patients with OSA are advised to use CPAP for the entire sleep period which for most of them is between 7 and 8 hours. Thus, people who adhere to this recommendation would, on average, spend a third of the 24-hour cycle with IOP 2-3 mmHg higher than it would be otherwise. This could potentially make a difference. For example, if we consider a patient with NTG whose pre-treatment IOP is 15mmHg. Target IOP is set on an individual basis but, in general, a 20% reduction would be considered an acceptable response, that is in this case 3 mmHg. CPAP could nullify this reduction making the treatment ineffective during the night. Another perspective on this is provided be epidemiological longitudinal studies, which show that lowering IOP by 1mmHg decreases the risk of glaucoma progression by 10% (260). Therefore, even small IOP changes may be important. In addition, as is often the case, average values do not tell the whole story. Complicating matters further is the variability of IOP elevation in POAG patients. Whilst in some patients IOP only increases minimally with CPAP, in others the increase is several times greater reaching values which may pose a more immediate risk to the optic nerve. As currently there is no practical way of checking nocturnal IOP during CPAP therapy outside the hospital setting, it is important to identify other factors associated with

significant IOP changes. This knowledge could help to determine which patients are at risk of potential complications from higher nocturnal IOP. The current study identified CPAP level as one of the predictors but there are probably others. Elucidating which of the potential mechanisms drives IOP elevation could also help in understanding the impact of CPAP on glaucomatous neuropathy.

*5.4.4 Changes in nocturnal OPP and BP*

Calculated OPP has long been used in glaucoma research as a surrogate marker of ocular perfusion. A large number of studies looking at its relevance to glaucoma development and progression have been published (261). The results have not been consistent probably in part because it is a crude marker of ocular perfusion and it is unclear how well it estimates the actual ocular blood flow. In addition, it is difficult to separate out the influence of IOP from the impact of blood pressure. Nevertheless, as CPAP treatment can potentially impact both components of OPP and in the absence of other tools to more precisely measure it at night, OPP was calculated in this study. In line with the data reported by Kiekens et al. in the current study OPP was lower on CPAP than at baseline in the control group. This represents the combined effects of IOP elevation and a non-statistically significant BP reduction. However, in the POAG group OPP has not changed as IOP elevation was counterbalanced by an unexpected increase in nocturnal BP.

Almost twice as many patients in the control group as in the POAG group had a diagnosis of essential hypertension and therefore CPAP could have had larger impact on BP in that group. Still, this would not explain why BP increased during CPAP therapy in POAG patients. Whether this is a type I error in a relatively small sample of patients or a true phenomenon indicating a differential effect of CPAP on BP in people with and without POAG requires further assessment in a larger sample, ideally with continuous beat-to-beat BP monitoring. The latter is however not inconceivable. Systemic autonomic dysfunction and vascular dysregulation have been documented in numerous studies and seem to be inherent features of POAG and are probably independent of OSA (262,263). Paradoxical autonomic responses to various provocation tests and metabolic stimuli have also been found in patients with POAG (264–266). Perhaps some of the seminal studies which assessed the impact of CPAP on endothelial dysfunction or sympathetic activation should be also carried out in people with POAG. At present, it is simply assumed that CPAP has the same beneficial effect on the cardio-vascular system in patients with POAG as it does in people without glaucoma but this may not be true.

*5.4.5 Study limitations*

The main limitation of this study is that participants were awakened for IOP and BP measurements. This is a common drawback in studies assessing nocturnal IOP but it should not affect pre- and post-CPAP comparisons. There is also some evidence that nocturnal hourly awakenings when compared with uninterrupted CLS measurements do not alter the mean variables of 24-hour IOP pattern (267).

Since the two previous studies showed no significant IOP changes during the day the focus of the current study was on assessing nocturnal IOP rather than the 24-hour IOP profile. Also, due to logistic difficulties and anticipated lower recruitment rate if participants were to be admitted for 24-hours, daytime IOP was self-measured by the participants at home. The device used for this (IcareHome) operates on exactly the same rebound technology as the one used for the nocturnal measurements (IcarePro) and has been previously validated against Goldmann tonometer(268). Nevertheless, as relatively large proportion of daytime IOP measurements were missing, the comparison of diurnal IOP is considered less robust.

Whilst the study was adequately powered for comparisons of the multiple repeated measurements, as discussed previously, the sample size may have been insufficient to detect small but potentially clinically relevant differences between the group in baseline characteristics.

The study assessed only patients with POAG and therefore the results are not generalisable to patients with other glaucoma subtypes or people with ocular hypertension in whom IOP control is also important. In addition, most of included POAG patients had mild visual field impairment which leaves uncertainty of how patients with more severe glaucoma would respond to CPAP. Due to small sample size it was not possible to examine the impact of different ocular hypotensive medications on CPAP related IOP changes. These are potential directions for future research.

Lastly, since polysomnography was not performed it is not possible to comment on what impact CPAP therapy had on sleep structure and sleep stages. Based on the oximetry monitoring an adequate control of OSA was achieved in the majority of patients, apart from two patients with severe OSA who could not tolerate higher levels of CPAP (in each the ODI has reduced by approximately 50% only from the pre-treatment baseline) and one other patient in whom CPAP probably induced complex sleep apnoea (the ODI has doubled with a characteristic oximetry pattern of central sleep apnoea).

## 5.5 Conclusions

This study provides further evidence that CPAP therapy leads to nocturnal IOP elevation. For the first time, this has been demonstrated in people with POAG. There was no overall

difference in IOP elevation between the groups (people with POAG and people without glaucoma) but patients with POAG had more variable responses. The IOP increase was not prevented by ocular hypotensive medications, although the impact of different therapies could not be established in this study. There are no reports that POAG is more common among CPAP users and it may be that these observations are only relevant to patients who already have or are at risk of developing optic neuropathy and require treatment for OSA. The mechanisms behind the IOP increase are unclear and it is yet to be determined whether IOP changes are CPAP specific or perhaps result from abolishing intermittent airway obstructions and the physiological consequences of this. The CPAP level does seem to be relevant. Ultimately, only longitudinal studies can determine whether CPAP treatment affects glaucoma progression. Until such data are available CPAP related IOP elevation should not be simply ignored. It will be appropriate, as a minimum, for sleep clinicians initiating patients with POAG on CPAP to liaise with our ophthalmology colleagues to ensure ongoing surveillance for deterioration in vision.

# Chapter 6: PAIR I study: Does intraocular pressure change in response to CPAP applied in wakefulness?

## Null hypothesis:

A short exposure to CPAP administered within the pressure range used in clinical practice does not cause an immediate IOP elevation in awake subjects.

### 6.1 Introduction

The study reported in the previous chapter demonstrated that CPAP increases IOP at night in both healthy subjects and in people with POAG. However, the mechanisms responsible for CPAP related IOP increase are unclear. One of the most commonly cited hypotheses is the mechanical pressure transmission from the upper airway through the intrathoracic space to the ocular venous system which would impede aqueous humour outflow via the episcleral veins (138,269–272). Data from physiological studies lend some support to this hypothesis. CPAP has been shown to increase intrathoracic pressure and right atrial pressure in a manner proportionate to the pressure level set on the machine (273–276). Reduction of venous return as a consequence of hydrostatic pressure changes is also believed to be responsible for the well documented IOP elevation when lying down. Upon assuming a supine posture both IOP and episcleral venous pressure (EVP) increase in parallel within a few minutes (277–279). The magnitude of these postural changes varies widely in the range of 1.6 to 8.6mmHg but several studies have found that, on average, it is greatest in people with glaucoma (280–284). Even slight elevation of the head by 20-30 degrees when lying down can alter IOP (285). Therefore, if CPAP raised IOP by a similar mechanism resulting in impaired drainage from the ocular venous system, one could not only expect prompt IOP changes shortly after CPAP application and irrespective of whether subjects are asleep or awake, but also that these changes are dose dependent. Following this mechanistic hypothesis, it could be further speculated that the magnitude of CPAP induced IOP change may be influenced by body weight or extra-thoracic restriction. The rationale for this assumption comes from observations that obese subjects develop intrinsic positive end-expiratory pressure (PEEPi) and have relatively high gastric and oesophageal pressures, particularly when supine (286). Glaucoma severity may also be a relevant factor. For instance, some studies found that orthostatic IOP changes correlate positively with the severity of visual field impairment in glaucoma (282,287).

Knowing the relationship between CPAP and IOP may be important from the clinical perspective as it could guide positive pressure titration, and perhaps inform the choice of positive pressure modality for people with OSA and concomitant POAG, in whom IOP elevation is likely to be particularly undesirable. In Chapter 3 it was demonstrated that nocturnal IOP changes after CPAP are more variable in people with glaucoma than in controls. In some patients the average increase in the overnight IOP was as high as 8 mmHg. It would probably be valuable to identify such patients early in their treatment pathway. Currently, in most sleep centres CPAP is initiated on an outpatient basis with the aid of automatically titrating devices (auto-CPAP). In comparison to an overnight manual pressure titration in a sleep laboratory environment, this model of care delivery has been shown to be cost-effective and non-inferior in the majority of long-term treatment outcomes (288,289). One opportunity to assess individual IOP responses to CPAP would be by measuring it during ambulatory CPAP set up visits. Clinicians could then decide on the maximum allowed CPAP level for patients with glaucoma or perhaps choose to treat those with high positive pressure requirements with a bi-level ventilator instead. The latter strategy allows the application of lower expiratory pressure, potentially reducing pressure transmission whilst still effectively controlling intermittent upper airway collapses (276). However, for this exercise to be valid we need to first understand whether a short-term diurnal application of CPAP can induce IOP changes of similar magnitude to those observed during nocturnal therapy and whether any potential changes are dependent on the CPAP level.

Therefore, the main aim of this study is to determine whether CPAP applied in wakefulness and set within a pressure range most commonly used in clinical practice alters IOP in people with and without glaucoma. Another goal is to examine whether any potential IOP changes correlate with the CPAP level and whether patients with treated and untreated POAG respond to CPAP differently. In addition, potential covariates of the hypothesised IOP change in response to CPAP are explored.

## 6.2 Methods

### *6.2.1 Participants and settings*

Three groups of participants were recruited: newly diagnosed treatment naïve POAG patients (group I), POAG patients established on treatment (group II), and control subjects without glaucoma (group III). Patients in group I were recruited from glaucoma clinics following their diagnosis of POAG and before they started IOP-lowering treatment. According to the study protocol the treatment could have not been delayed by participation in the study for more than 4 weeks and if more urgent therapy was indicated based on clinical discretion, the potential participants were not approached. Participants

to group II and III were recruited among patients who had previously taken part in the POSAG study.

All participants were over 40 years old and had not used CPAP before. They all had a prior comprehensive ocular examination which consisted of the following tests: automated visual fields (HFA, SITA-Fast, Carl Zeiss Meditec, Jena, Germany), slit-lamp biomicroscopy and gonioscopy (Haag-Streit International, Koeniz, Switzerland), Goldmann applanation tonometry (Haag-Streit International, Koeniz, Switzerland), central corneal thickness measurement (Pachmate 2, DGH Technology Inc., Exton, USA), fundus photography and SD-OCT (Heidelberg Engineering Inc. MA, USA). As part of the POSAG study patients in groups II and III were previously investigated with an rPG sleep study. OSA status was unknown for participants in group I.

The exclusion criteria were as follows:

- previous surgical treatment for glaucoma
- current or recent (within 4 weeks) CPAP or non-invasive ventilation use
- history of face mask intolerance
- contraindications to rebound tonometry (including: corneal scarring, microphtalmos, buphthalmos, nystagmus, keratoconus, abnormal central corneal thickness, corneal ectasia, active corneal infection)
- concomitant eye diseases known to affect IOP (including: wet ARMD, CRVO, BRVO, uveitis and diabetic retinopathy)
- significant lung diseases (including: previous pneumothorax, previous or current respiratory failure of any aetiology, chronic obstructive pulmonary disease, bullous lung disease, difficult to control asthma, acute chest infection)
- significant cardiac diseases (including: heart failure, unstable arrhythmias, pulmonary hypertension).

The study was conducted in a tertiary sleep centre at the Royal Papworth Hospital in collaboration with the Eye Clinic at Hinchingbrooke Hospital, North West Anglia Foundation Trust, UK. Ethical approval was given by the East of England – Cambridge South Research Ethics Committee (REC number 16/EE/0073) and the Anglia Ruskin University Research Ethics Panel (ref. 16/17 011) (Appendix 20). The tenets of the Declaration of Helsinki were followed. Written informed consent was obtained from all participants.

### *6.2.2 Study procedures*

Following a written informed consent all subjects underwent upright spirometry and baseline anthropomorphic measurements. IOP was first measured in a sitting position and again 30 min later in a supine position. Whilst IOP increases within a few minutes, the 30 min time interval was chosen for consistency with the majority of previous studies

examining orthostatic IOP fluctuations, and to allow sufficient time for IOP to stabilise before application of CPAP (279). A NIPPY 3+ ventilator (Breas Medical) was used to deliver CPAP. This ventilator alarms instantly in case of significant air leaks which allows the operator to immediately adjust the mask. In addition, prior to each set of repeated measurements the circuit pressure was monitored using a manometer with a pressure transducer in line between the mask and the tubing.

All participants were fitted with a full face mask. CPAP was set at four pressure levels: 6, 10, 13 and 16cmH2O which were administered in a random order to eliminate CPAP exposure time as a confounder (Table 6-1). CPAP level sequences were computer generated in R (version 3.4.1) and randomly allocated to consecutive participants. They were provided to the investigator in sealed envelopes after consenting. Each CPAP level was applied for 30 min. IOP was checked repeatedly in both eyes using a rebound tonometer (IcarePro) which allows the user to obtain accurate measurements in both sitting and supine positions without interrupting CPAP administration. The person obtaining IOP measurements was blinded to the CPAP level. BP and SpO2 were checked repeatedly at each CPAP level and prior to IOP measurements. Participants remained lying down in a supine position for a total of 2.5 hours. The head end of the bed was raised up to 20° and one thin pillow was allowed for comfort (Appendix 21). Once all measurements were completed, participants were provided with an eye lubricant to use at home in case of symptoms of dry eyes.

Table 6-1. Illustration of repeated measurements sequence with an example of randomly allocated CPAP level order.

| Time/CPAP/Position | SpO2 | BP | IOP right and left eye |
|---|---|---|---|
| 0 min/no CPAP/upright | ✓ | ✓ | ✓ |
| 30 min/no CPAP/supine | ✓ | ✓ | ✓ |
| 60 min/16 cmH2O/supine | ✓ | ✓ | ✓ |
| 90 min/10 cmH2O/supine | ✓ | ✓ | ✓ |
| 120 min/13 cmH2O/supine | ✓ | ✓ | ✓ |
| 150 min/6 cmH2O/supine | ✓ | ✓ | ✓ |

Abbreviations: CPAP=continuous positive airway pressure, SpO2=oxygen saturation, BP=blood pressure, IOP=intraocular pressure.

### *6.2.3 Study outcomes*

*Primary:*

Change in IOP after application of CPAP (difference between baseline supine IOP and IOP on CPAP).

*Secondary:*

i. Differences in IOP change (ΔIOP; $IOP_{CPAP}$ -$IOP_{baseline}$) between the groups.
ii. Minimum CPAP level which leads to IOP increase in each group.
iii. Correlation of IOP change (ΔIOP) in response to CPAP with: CPAP level, OSA severity (AHI), BMI, %predicted FVC.
iv. BP and SpO2 changes in response to CPAP across the groups.

### *6.2.4 Statistical considerations*

#### *6.2.4.1 Sample size*

This was an exploratory study and there were no reliable published data to enable an accurate sample size calculation for repeated comparisons. Nevertheless, IOP variability among patients with and without POAG was known from the POSAG study and certain assumptions could be made in addition, to guide the minimum required sample size. The mean±sd postural IOP increase in 398 POSAG patients was 1.5±2.0mmHg ($F=190.5$, $p=0.000$) with a calculated partial effect size of 0.32 (partial $\eta 2$). To compute the required sample size for repeated measures model with 5 repetitions per subject, it was assumed that the same proportion of within group variance in IOP could be explained by the effect of CPAP as it was by the posture change in POSAG. Another assumption was that a correlation among repeated IOP measurements is 0.5. Based on this, and provided there were no missing data, only 6 patients per group would be required to detect IOP difference on any CPAP level with 95% power and 5% significance. However, to account for incomplete measurements from subjects who could not tolerate higher CPAP levels and also to allow subsequent subgroup analyses, including multivariate analyses in case several variables were found to predict IOP response to CPAP, the study aimed to recruit up to 30 participants per group. An interim analysis was planned after recruiting at least 50% of all subjects which would allow early termination of recruitment if there was no indication that significant differences in IOP could be detected within the originally planned maximum sample size. The sample size was calculated in G*Power, version 3.0.10 (Appendix 22).

*6.2.4.2 Statistical analysis*

Continuous data are presented as mean (SD) or median (IQR) depending on the distribution. Categorical data are reported as percentages. Comparisons of baseline characteristics between the groups were performed using one-way ANOVA with post hoc Bonferroni tests if significant differences were detected. Assumptions of normality and equality of variance were assessed using the Shapiro-Wilk and Levene's homogeneity of variance tests, respectively. If either of these assumptions was violated, or in case of categorical data, the Kruskal-Wallis H test was used.

A mixed-model ANOVA with CPAP level (supine off CPAP, 6, 10,13 and 16cmH2O) and eye (right and left) as within-subjects factors was built to assess IOP responses to CPAP. For between group comparisons of CPAP related IOP change, ΔIOP, defined as: $IOP_{CPAP}$-$IOP_{supine}$, was calculated for each CPAP level and plotted as a within-subject factor. This allowed for anticipated baseline differences in IOP to be accounted for between the groups. Post hoc pairwise comparisons with Bonferroni correction were performed for any variables with a significant within-subject effect and interactions. Sphericity was evaluated and in cases where sphericity was violated, the sphericity-corrected P value is reported. Pearson test was used to examine correlations between ΔIOP and potential linear co-variates. The level of significance for each comparison was set at $p < 0.05$. All analyses were conducted using SPSS software version 22.0 (IBM SPSS, IL, USA).

## 6.3 Results

*6.3.1 Included participants*

Following an interim analysis, the Trial Steering Committee took a decision to terminate the study prematurely. This was dictated by meeting the pre-defined termination criteria for the primary outcome. At that point a total of 46 participants (51% of the original sample size) had been enrolled. This included: 8 untreated POAG patients, 21 treated POAG patients and 17 controls. Tables 6-2 and 6-3 describe baseline characteristics of included participants.

Table 6-2. Baseline characteristics of participants who took part in PAIR I study (one-way ANOVA or Kruskal-Wallis H test).

| | Untreated POAG N=8 | Treated POAG N=21 | Controls N=17 | P value |
|---|---|---|---|---|
| Age (yr), sd | 62±11.6 | 71±4 | 70.3±3.5 | 0.16 |
| Sex (% Male) | 75 | 67 | 47 | 0.32 |
| BMI (kg/m2),sd | 25.5 ±.4.4 | 27.1±3.3 | 27.3±4 | 0.71 |
| Neck (cm),sd | 38.3±2.6 | 39.7±4.2 | 38.9±4.2 | 0.4 |
| FEV1 predicted (%), sd | 103±20 | 102±15 | 100±0.14 | 0.87 |
| FVC predicted (%), sd | 105±13 | 102±14.3 | 102±12.4 | 0.65 |
| FEV1/FVC | 0.78±0.1 | 0.78±0.1 | 0.77±0.1 | 0.95 |
| Antihypertensive Medications (%) | 37.5 | 33.3 | 52.9 | 0.46 |

Abbreviations: BMI=body mass index, FEV1=forced expiratory volume in one second, FVC=forced vital capacity, POAG=primary open-angle glaucoma.

Table 6-3. Ocular characteristics of participants with POAG who took part in PAIR I study.

| | Right Eye | Left Eye |
|---|---|---|
| *Untreated POAG* | | |
| MD (dB), IQR | -1.6±3.1 | -1.9±3.8 |
| VFI, (%), IQR | 97.5±5 | 94±8 |
| Highest ever recorded IOP, (mmHg), sd | 21.8±4.2 | 22.8±4.1 |
| CCT, sd | 543±21.9 | 541±20.6 |
| *Treated POAG* | | |
| MD (dB), IQR | -2.1±3.4 | -5±8.4 |
| VFI, (%), IQR | 96.5±6 | 88.5±26 |
| Highest ever recorded IOP, (mmHg), sd | 21.1±3 | 21.2±3.5 |
| CCT, sd | 541.4±24 | 541±18.5 |
| Topical treatment (%): | | |
| Prostaglandin analogue | 71.4 | 76.2 |
| Beta blocker | 38.1 | 42.9 |
| Carbonic anhydrase inhibitor | 28.6 | 38.1 |
| Alpha agonist | 9.5 | 9.5 |
| Cholinergic agonist | 4.8 | 4.8 |

Abbreviations: MD=mean deviation, VFI=visual field index, CCT=central corneal thickness, IOP=intraocular pressure, POAG=primary open-angle glaucoma.

*6.3.2 Postural IOP changes*

Baseline IOP measured in an upright position was significantly higher in the untreated POAG group than in the two other groups (Table 6-4). There was no difference in IOP between the treated POAG group and the control group which is in keeping with adequately controlled IOP in the former. Upon assuming a supine position IOP increased by a mean of 1.8±2 mmHg across the 3 groups (F=27.3, p=0.000). The magnitude of positional IOP change was not significantly different between the groups (F=1.23, p=0.32, Table 6-5).

Table 6-4. Baseline upright IOP across the groups (one way ANOVA).

| | Untreated POAG-I | Treated POAG-II | Controls-III | P value |
|---|---|---|---|---|
| Right Eye, mmHg, sd | 19.2±3 | 15.2±2.8 | 15.8±2.7 | 0.005* |
| Left Eye, mmHg, sd | 19.1±3.4 | 15.4±3 | 15.8±2.8 | 0.017† |

Abbreviations: IOP=intraocular pressure, POAG=primary open-angle glaucoma.

*I vs II p=0.004; I vs III p=0.023; II vs III p=1

† I vs II p=0.016; I vs III p=0.049; II vs III p=1

Table 6-5. Postural IOP change across the groups (mixed model ANOVA).

| | Untreated POAG-I | Treated POAG-II | Controls-III | P value |
|---|---|---|---|---|
| $IOP_{upright}$-$IOP_{supine}$, mmHg, sd | -1.4±2 | -2.3±1.8 | -1.3±2.2 | 0.32 |

Abbreviations: IOP=intraocular pressure, POAG=primary open-angle glaucoma.

*6.3.3 Primary Outcome: IOP changes in response to CPAP*

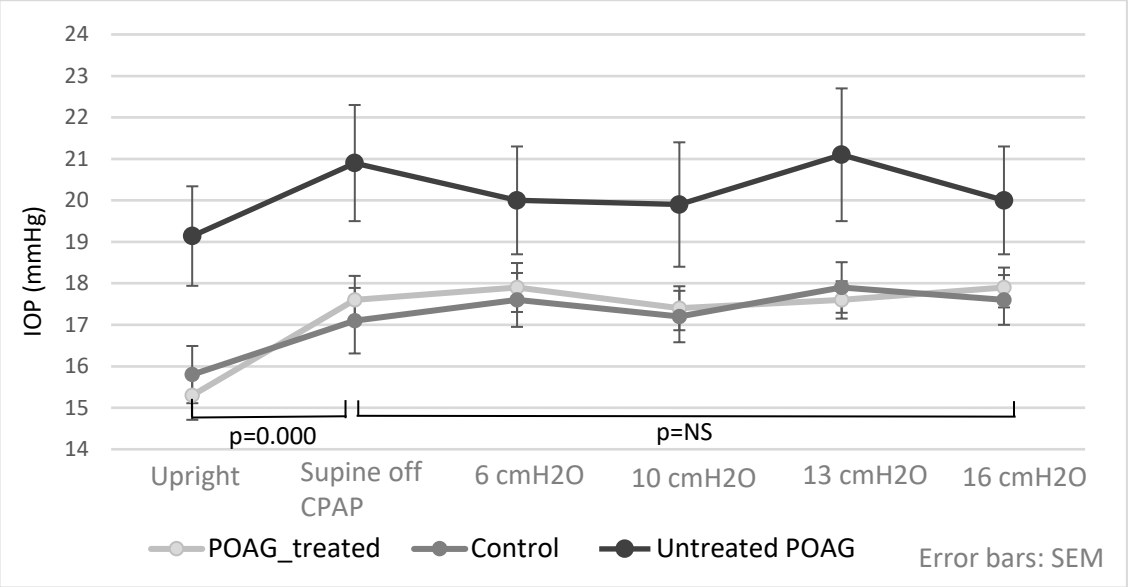
Following application of CPAP, there was no significant change in IOP across all CPAP levels and in relation to the supine measurements off CPAP (n=44; $IOP_{supine}$: 17.9±3.2mmHg, $IOP_{6cmH2O}$: 18.1±2.8mmHg, $IOP_{10cmH2O}$: 17.7±2.8mmHg, $IOP_{13cmH2O}$: 18.3±3.3mmHg, $IOP_{16cmH2O}$: 18.1±2.9mmHg, F=1.2, p=0.35), see Figure 8. The three groups did not differ in their responses to CPAP ($\Delta IOP_{CPAP\text{-}Supine;}$ group I: -0.69±1.4mmHg, group II: 1.2±1.4mmHg, group III: 0.44±1.4mmHg, F=1.6, p=0.21) (Figure 6-1).

In order to check whether the duration of CPAP exposure was important, an additional analysis was conducted. In this analysis, time rather than CPAP level was used as within-

subject factor. The results indicated that IOP remained fairly constant during the two hours of CPAP application (n=44, $IOP_{supine}$: 17.9±3.3mmHg, $IOP_{30min}$: 18±3.1mmHg, $IOP_{60min}$: 17.9±2.8mmHg, $IOP_{90min}$: 18.1±2.9mmHg, $IOP_{120min}$: 18.2±3.3mmHg, F=0.48, p=0.75). The actual frequencies of each CPAP level at each time point were not statistically different from the expected frequencies confirming that randomisation of CPAP level sequence was successful (Table 6-6).

The circuit pressure measurements taken on expiration at the CPAP mask just prior to IOP measurements indicated good control of air leaks (Table 6-7 and Figure 6-2).

Figure 6-1. IOP response to posture change and CPAP in PAIR I study (repeated measures ANOVA).




Abbreviations: IOP=intraocular pressure, POAG=primary open-angle glaucoma, NS=not statistically significant, SEM=standard error of mean.

Table 6-6. CPAP sequence randomisation-distribution of frequencies (%) of each CPAP level in time order.

| CPAP level | Time 1 (30 min) | Time 2 (60 min) | Time 3 (90 min) | Time 4 (120 min) |
|---|---|---|---|---|
| 6 cmH2O | 15.2 | 28.3 | 28.3 | 28.3 |
| 10 cmH2O | 28.3 | 30.4 | 15.2 | 26.1 |
| 13 cmH2O | 34.8 | 10.9 | 30.4 | 23.9 |
| 16 cmH2O | 21.7 | 30.4 | 26.1 | 21.7 |
| p value* | 0.27 | 0.18 | 0.47 | 0.93 |

Abbreviations: CPAP=continuous positive airway pressure.

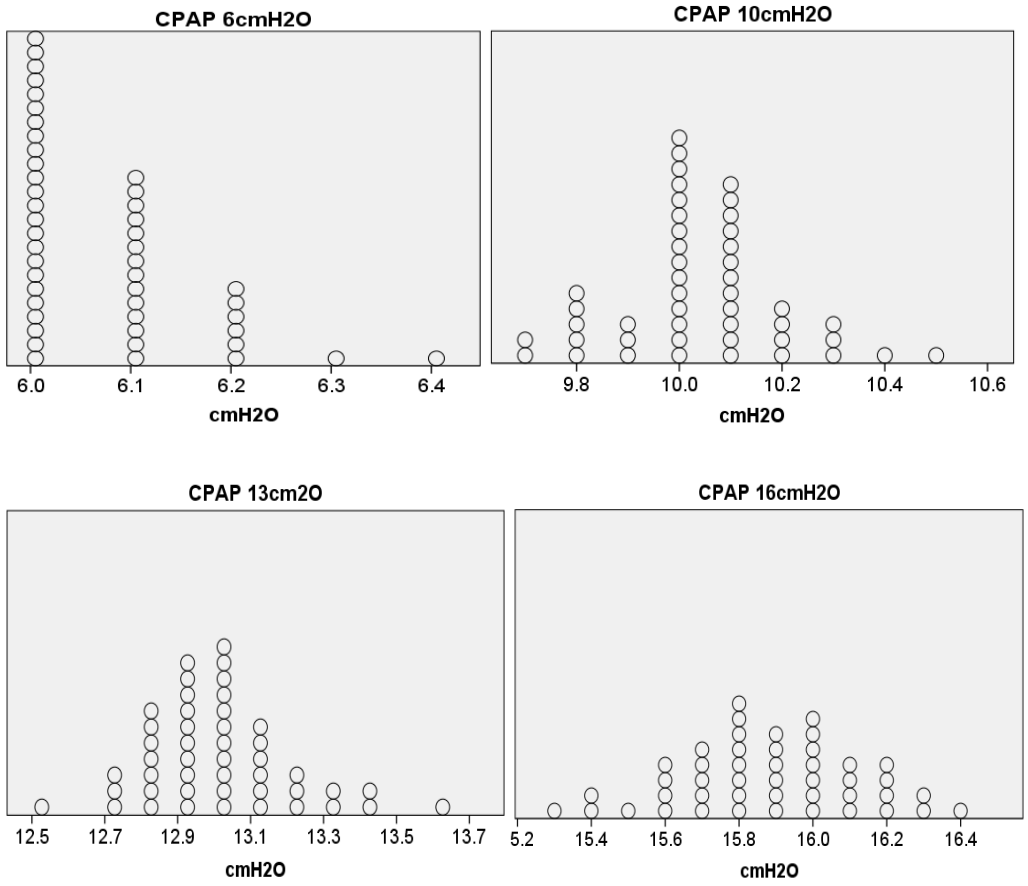
*Chi-square test

Table 6-7. Circuit pressure measured at the mask on each CPAP level.

| CPAP level set (cmH2O) | 6 | 10 | 13 | 16 |
|---|---|---|---|---|
| Actual circuit pressure, (cmH2O), IQR | 6±0.1 | 10±0.1 | 13±0.2 | 15.9±0.4 |

Abbreviations: CPAP=continuous positive airway pressure.

Figure 6-2. Histograms showing distribution of positive pressured measured in the circuit for each CPAP level.




Abbreviations: CPAP=continuous positive airway pressure.

*6.3.4 Subgroup analyses*

To examine whether IOP behaves differently in response to CPAP in people with OSA, excessive weight, and more severe glaucoma the following subgroup analyses were conducted.

*OSA vs no OSA*

OSA status was known for the treated POAG group and the control group. In these two groups, 23 out of 38 participants were diagnosed with OSA (mild: n=15; moderate: n=7; severe: n=1). IOP change after exposure to CPAP was not significantly different between people with and without OSA (ΔIOP; OSA group: 0.53±1.3mmHg, no OSA group: -0.13±1.3mmHg, F=2.3, p=0.14).

*BMI*

Participants were categorised according to their BMI into 3 groups: 1. BMI<25kg/$m^2$(n=12), 2. BMI ≥25 to <30 kg/m2 (n=22) and 3. BMI≥30kg/m2 (n=10). There was no between group difference in IOP change after exposure to CPAP (ΔIOP; group 1: -0.25±1.4mmHg, group 2: 0.24±1.4mmHg, group 3: 0.25±1.4mmHg, F=0.54, p=0.59).

*Glaucoma Severity*

The severity of glaucoma was determined based on visual field defect in the worse affected eye. First, each patient with POAG was categorised into one of seven stages of glaucoma severity according to the classification by Brusini (enhanced Glaucoma Severity Staging system-eGSS) which was described in Chapter 3 (164). Then, a consolidated glaucoma staging system proposed by Ng et al. was used (290). According to the latter classification there are 4 stages of glaucoma: CS1-Normal (eGCS-stage 0 and Border), CS2-Mild (eGCS-stage 1), CS3-Moderate (eGCS-stage 2 and 3), CS4-Severe/End-Stage defect (eGCS-stage 4 and 5). We identified 11 participants in CS1 (median MD: -1.8±1dB), 11 in CS2 (median MD: -5.7±2.7dB) and 6 in CS3 (median MD: -11.8±5.7dB). There were no patients with severe/end-stage defect.

When the three stages of glaucoma severity were compared, there was no significant between group difference in IOP change in response to CPAP (ΔIOP; CS1: 0.9±1.5mmHg, CS2:0.36±1.5mmHg, CS3: -1.2±1.4mmHg, F=2.3, p=0.12).

Further, there were no correlations between IOP change on the highest level of CPAP (ΔIOP; $IOP_{16cmH2O}$-$IOP_{supine}$) and: AHI (n=37, Right eye: r=0.03,p=0.84; Left eye: r=-0.09, p=0.59), BMI (n=45, Right eye: r=0.19,p=0.2; Left eye: r=-0.013, p=0.93), predicted FVC (n=45, Right eye: r=-0.09,p=0.55; Left eye: r=-0.11, p=0.47) and MD (n=29, Right eye: r=-0.09,p=0.65; Left eye: r=0.26, p=0.17).

*6.3.5 Changes in vital signs*

Both SBP and DBP decreased significantly after participants rested in a supine position for 30 minutes: 133.2 vs 127mmHg (p=0.00, paired t-test), 77.4 vs 74.2mmHg (p=0.011, paired sample t-test), respectively. SpO2 remained unchanged (96.1±1.8% vs 96±1.9%, p=0.67).

Application of CPAP led to an increase in SBP and DBP which was significant for nearly all CPAP levels (Table 6-8). No differences in the magnitude of SBP and DBP change were detected between the groups ($\Delta SBP_{CPAP\text{-}Supine;}$ group I: 4.9±11.3mmHg, group II: 5.1±11.5mmHg, group III: 6.1±11.2 mmHg, F=0.05, p=0.96, $\Delta DBP_{CPAP\text{-}Supine}$; group I: 5.5±5.4mmHg, group II: 6.9±5.5 mmHg, group III: 5.1±5.4 mmHg, F=0.56, p=0.58). CPAP increased SpO2 in a dose dependent manner across the groups (Figure 6-3).

Table 6-8. Vital signs according to CPAP level (repeated measures ANOVA).

| | Off $CPAP_{Supine}$ | $CPAP_{6cmH20}$ | $CPAP_{10cmH20}$ | $CPAP_{13cmH20}$ | $CPAP_{16cmH20}$ | p value |
|---|---|---|---|---|---|---|
| SBP, mmHg, sd | 127.3±15.4 | 132.7±14.1 | 132.8±14.1 | 134.6±13.4 | 132.8±14.8 | 0.010* |
| DBP, mmHg, sd | 74.5±8.7 | 80.2±8.3 | 78.1±7.9 | 81.4±8 | 82.5±6.8 | 0.000† |
| SpO2, %, sd | 96±1.9 | 97.0±1.8 | 97.4±1.6 | 97.6±1.2 | 97.7±1.4 | 0.000[&] |

Abbreviations: CPAP=continuous positive airway pressure, SBP=systolic blood pressure, DBP=diastolic blood pressure, SpO2=oxygen saturation.

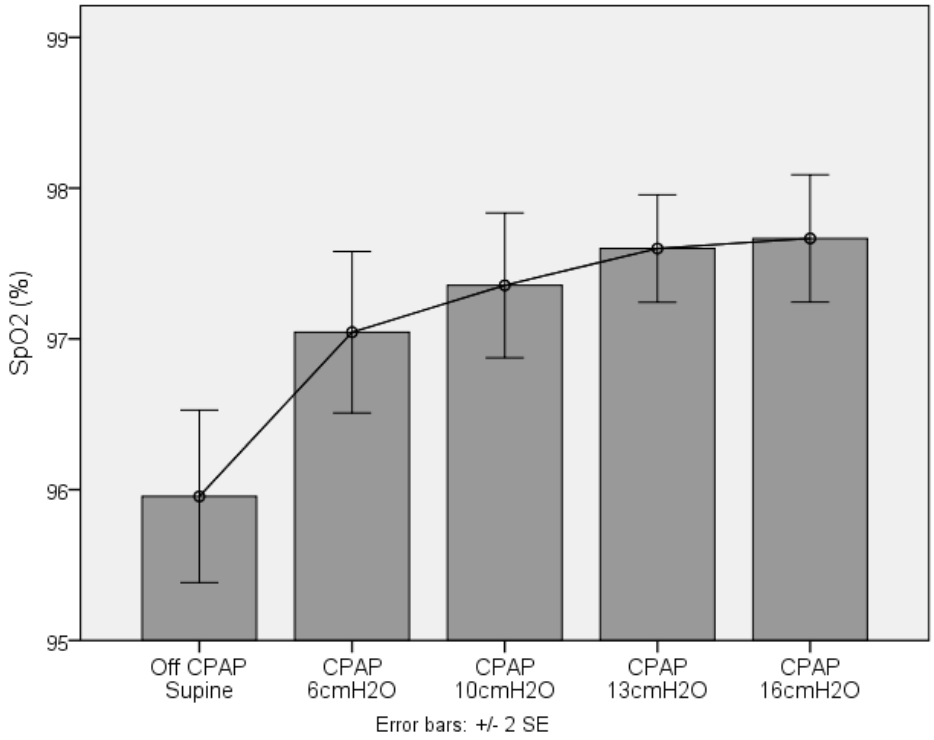
For all comparisons: n=45.

* Off $CPAP_{Supine}$ vs $CPAP_{6cmH20}$, p=0.049; Off $CPAP_{Supine}$ vs $CPAP_{10cmH20}$, p=0.017; Off $CPAP_{Supine}$ vs $CPAP_{13cmH20}$, p=0.003; Off $CPAP_{Supine}$ vs $CPAP_{16cmH20}$, p=0.098

† Off $CPAP_{Supine}$ vs $CPAP_{6cmH20}$, p=0.000; Off $CPAP_{Supine}$ vs $CPAP_{10cmH20}$, p=0.011; Off $CPAP_{Supine}$ vs $CPAP_{13cmH20}$, p=0.000; Off $CPAP_{Supine}$ vs $CPAP_{16cmH20}$, p=0.000; $CPAP_{10cmH2O}$ vs $CPAP_{13cmH20}$, p=0.024; $CPAP_{10cmH2O}$ vs $CPAP_{16cmH20}$, p=0.003.

[&] Off $CPAP_{Supine}$ vs $CPAP_{6cmH20}$, p=0.001; Off $CPAP_{Supine}$ vs $CPAP_{10cmH20}$, p=0.000; Off $CPAP_{Supine}$ vs $CPAP_{13cmH20}$, p=0.000; Off $CPAP_{Supine}$ vs $CPAP_{16cmH20}$, p=0.000; $CPAP_{6cmH2O}$ vs $CPAP_{16cmH20}$, p=0.025.

Figure 6-3. Changes in oxygen saturation in relation to CPAP level.




Abbreviations: SpO2= oxygen saturation, CPAP=continuous positive airway pressure, SE=standard error.

*6.3.6 Missing data*

One control patient could not tolerate the highest level of CPAP and, as such, IOP measurements could not be performed on this CPAP level. In addition, despite several attempts we could not perform reproducible IOP measurements in one eye for one untreated POAG patient on a CPAP level of 10cmH2O. Therefore, there were 3 missing IOP variables out of 460 used in the analysis of the primary outcome. As the ANOVA test does not tolerate any missing data those two patients were excluded by default from the repeated measures model. However, in a subsequent sensitivity analysis the missing variables were substituted with IOP readings obtained on a prior CPAP level for each patient. This allowed inclusion of both subjects into the analysis with no important changes to any of the reported models. For instance, for the primary outcome model mean IOP values and the p value remained practically unaltered (n=46; $IOP_{supine}$: 17.9±3.2mmHg, $IOP_{6cmH2O}$: 18.0±2.8mmHg, $IOP_{10cmH2O}$: 17.7±2.8mmHg, $IOP_{13cmH2O}$: 18.32±3.3mmHg, $IOP_{16cmH2O}$: 18.1±2.9mmHg, F=1.2, p=0.32).

## 6.4 Discussion

### *6.4.1 Summary of the main findings*

This study challenges the concept of mechanical positive airway pressure transmission as a predominant mechanism behind IOP elevation during CPAP therapy. IOP is a dynamic parameter and can change in a matter of seconds to minutes. If IOP increase was caused by impaired drainage from the ocular venous system, one would expect prompt IOP changes after CPAP application, similar to those occurring during changes in posture. In line with well-established evidence, in this study IOP also increased significantly across all three groups upon assuming a supine position. However, there were no further IOP changes after CPAP administration for up to 2 hours. This is despite application of positive pressure of up to 16cmH2O which is considered to be moderate to high in clinical practice of OSA treatment.

It could be argued that only certain patients are at risk of IOP increase from exposure to CPAP and that the effect size could have been diluted by analysing data in the whole cohort of participants. To investigate this, the three groups of participants were compared and additional subgroup analyses were performed which indicated that glaucoma status, topical medications used to lower IOP, BMI, OSA status and glaucoma severity were not important factors in IOP response to CPAP.

### *6.4.2 Physiological interpretation of the main results*

Posture induced EVP changes are believed to be directly responsible for IOP elevation in the supine position. It is unknown whether CPAP raises EVP but it is likely that, even if it does, any potential changes would be of a lesser magnitude than those observed during orthostatic fluctuations. In the supine position venous pressure in the ocular vessels is close to central venous pressure measured at the right atrium which, in euvolaemic subjects, nearly doubles upon lying down (291,292). Elevation of CVP is the driving force for EVP increase although the corresponding changes in EVP seem smaller, ranging between 0.8 to 3.6 mmHg, and this is likely related to a tonic neural control present in the episcleral circulation (277,278,293,294). CPAP also increases right atrial pressure but to a lesser degree. Studies which examined the haemodynamic consequences of PAP indicate gradual pressure loss on this upstream pathway from the upper airway to the right atrium. For instance, Becker et al. who applied three levels of CPAP: 5,10 and 15cmH2O, showed a linear increase in end-expiratory oesophageal pressure with an approximate transmission ratio of 2:1 (276). Van den Berg et al. demonstrated during gradual titration of PEEP from 0 to 20cmH2O that, on average, only 32±20% of the increase in airway pressure was translated into the increase in right atrial pressure (273). Thus, in healthy subjects the impact of CPAP on haemodynamic

parameters relevant to IOP regulation seems smaller than the impact of gravity observed during the postural changes.

Another possible route of CPAP transmission discussed in the literature is via the intracranial space. Raised intrathoracic or intraabdominal pressure can be transmitted into cerebro-spinal fluid (CSF) system through the dural venous sinuses, epidural venous plexus and probably to some degree directly via the intervertebral foramina (295,296). Increased CSF pressure could then raise IOP via the lamina cribrosa. It has been well established that the Valsalva manoeuvre elevates CSF pressure (297). However, the corresponding IOP changes are several times smaller. For example, in a study by Zhang et al. CSF pressure increased by 10.5 mmHg but IOP only by 1.9mmHg during the Valsalva manoeuvre (298). Whether CPAP is capable of altering CSF pressure is still unclear. Studies using the same level of PAP but in different groups of patients yielded contradictory results (299–301). Nevertheless, the amplitude of any potential CPAP related changes would likely be much smaller than during forced expiratory manoeuvres and thus have minimal impact on IOP. Furthermore, if any IOP changes occurred via this hypothetical transmission route they would have been detected in the time frame of the current experiment.

In summary, whilst there is little doubt that very high intrathoracic pressure, such as that generated during the Valsalva manoeuvre, will inevitably increase IOP irrespective of the pressure transmission route, the CPAP levels used to treat OSA do not seem high enough to directly raise IOP in a short space of time and in absence of other factors related to sleep (302,303).

*6.4.3 Comparison with previous studies*

To my knowledge only one previous study sought to examine a direct impact of CPAP on IOP in awake subjects (272). In that study 18 patients with glaucoma and 22 subjects without glaucoma were exposed to 12cmH2O of CPAP administered via a nasal mask for 15 min. IOP was measured with a Goldmann tonometer in an upright position immediately after removing the mask. The authors reported that IOP increased by an average of 2 mmHg only in patients with glaucoma. On this basis, they advocated caution when using CPAP in patients with confirmed or suspected glaucoma. The contradictory results of the current study in the glaucoma groups, may be explained by more robust design which allowed several biases to be eliminated and methodological limitations of the previous study. Firstly, in the current experiment patients used CPAP for a longer period and were lying down during the measurements which is the usual position assumed by CPAP users. As the gradient for venous return from the upper body

decreases in the supine position the additional impact of raised intrathoracic pressure from CPAP on venous return and already relatively high IOP may be different. Secondly, applying CPAP via a full face rather than a nasal mask meant that air leaks were minimised. The absence of a significant loss of positive pressure was confirmed by measuring it directly in the circuit. The measurements were performed during continues application of CPAP rather than directly after removing the mask. Thirdly, as opposed to using Goldmann tonometry which, as already discussed, is a largely operator dependent technique, in the present study IOP was measured with IcarePro reducing the risk of operator dependent bias. Furthermore, the ophthalmologist taking the measurements was blinded to the CPAP level applied. Fourthly, the study not only examined a wider group of patients, including those with untreated POAG who would be potentially more susceptible to any factors altering aqueous outflow, but also used several levels of CPAP, with the highest two being greater than the single CPAP level selected in the previous experiment. Whilst the sample size of the current study was only slightly larger, performing repeated measurements for each patient meant that the study power is likely to be substantially higher giving more confidence in the obtained results.

*6.4.4 Changes in vital signs*

As could be expected, CPAP increased oxygen saturation. This is likely a direct result of increased functional residual capacity and alveolar recruitment well described after CPAP application (304).

Persistent elevation of blood pressure was not anticipated but is in keeping with findings of another recent study in which the authors examined the acute impact of CPAP titration on a beat-to-beat blood pressure in awake subjects (305). They found significant increases in SBP and DBP which were correlated with incremental CPAP levels. In the present study the effect of CPAP on blood pressure was not dose dependent but this may be due to only intermittent blood pressure monitoring as opposed to continuous beat-to-beat recording which better reflects any haemodynamic changes. It should also be noted that, in the current study, each CPAP level was applied for 30 min rather than only for 3 min as in the previous study. Thus participants had more time to acclimatise to changing CPAP levels. Still, one possible explanation for the blood pressure increase is anxiety and stress associated with using CPAP by participants who had no prior experience with it. However, as the blood pressure remained elevated for up to 2 hrs and participants otherwise appeared relaxed while resting in a recumbent position, it may be that other factors, perhaps related to a direct sympathetic activation from raised intrathoracic pressure, play a role. Whether psychological stress also increases IOP is a matter of debate. There is no clearly established evidence base for this but a few studies

indicated a small elevation in IOP associated with mental stress (306–308). The possible mechanisms include dilatation of the pupil, tension of intraocular muscles and activation of glucocorticoid and catecholaminergic pathways. Nevertheless, if the blood pressure changes observed in our study were indeed caused by a stress reaction, it was not severe enough to influence IOP.

### *6.4.5 Limitations*

The participants in this study remained awake throughout the experiment. This is considered as a limitation in examining factors which may be important in sleep. These factors were discussed in Chapter 3.

Oesophageal, right atrial and episcleral pressures were not measured. Therefore, it is unclear to what extend CPAP altered intrathoracic and venous pressure at each step of this hypothesised upstream pathway. Whilst such measurements could further our understanding of this problem, it is technically challenging to perform all of them simultaneously and they involve invasive techniques with a significant risk of adverse events in an experiment with human subjects. Incorporating these measurements a priori without first establishing whether IOP actually increases after application of CPAP, would be ethically questionable and counterintuitive. The observation that SpO2 levels were higher during CPAP application would indicate that both airway and intrathoracic pressure have increased. The study did not examine a full range of CPAP level used in OSA treatment. Therefore, it is still possible that CPAP set above 16cmH20 raises IOP. The average CPAP level used for chronic treatment of OSA varies slightly depending on the titration process and the mode of CPAP used but it is in the range of 10-12cmH2O (309). The maximum recommended pressure level is 20cmH2O but CPAP above 15cmH2O is rarely required and can be poorly tolerated. The American Academy of Sleep Medicine guidelines recommend consideration of switching CPAP to a bi-level ventilation if pressure above 15cmH2O is reached (310). The additional concern about using a higher CPAP level in this study was that CPAP naïve patients might struggle to cope with such high pressure and it would have been more difficult to control air leaks. Thus the choice of the CPAP level range was pragmatic but is also believed to be representative.

It cannot be excluded that longer application of CPAP even in awake subjects alters IOP. A longer duration study could be considered but it would be more challenging to carry it out due to the commitment required from participants to remain in a supine position without interrupting CPAP application. Also, one would have to account for individual circadian IOP variations if the experiment went on for substantially longer.

### 6.5 Conclusions

In conclusion, this study demonstrates that application of CPAP for up to 2 hours in awake subjects does not cause significant changes in IOP. Therefore, measuring IOP during a brief CPAP exposure in office hours is not going to be a useful test to predict individual IOP responses to nocturnal CPAP therapy.

The situation is different at night when OSA subjects intermittently and repeatedly obstruct their upper airway when asleep. The physiological changes from re-establishing upper airway patency may be more important to the IOP alteration. CPAP transmission may still play a role in combination with other factors but is less likely to be a sole mechanism of IOP elevation at night. Indeed, a combination of: mechanical pressure transmission, elimination of intrathoracic pressure swings, changes in extracellular fluid level and possibly other factors, could be what leads to IOP increase. Future studies which set to investigate the potential mechanisms of CPAP related IOP elevation should take into account that sleep is essential to observe the relationship between CPAP and IOP.

# Chapter 7: Does a short course of CPAP therapy in people with OSA cause changes in ocular microvasculature? A pilot study.

## Null hypothesis:

A short course of CPAP in people with OSA does not alter the optic disc and peripapillary blood vessel density measured by OCT angiography in people with OSA with and without glaucoma.

### 7.1 Introduction

Ocular blood flow has long been implicated in the pathogenesis of glaucomatous neuropathy. According to the 'vascular' theory impaired ocular blood flow, particularly at night, leads to relative ischaemia which damages the optic nerve (29). Various tools have been used to assess vascular dysregulation in glaucoma; from simple formulae based on brachial artery pressure and IOP which can merely estimate ocular perfusion pressure to sophisticated and complex techniques such as laser Doppler flowmetry and laser speckle flowgraphy which require substantial expertise in acquisition of the measurements and thus face the problem of reproducibility and generalisability to clinical practice (311). The additional limitation of these technologies is the lack of direct visualisation of ocular microvasculature and inability to separate different vascular beds supplying the optic nerve and the retina. OCT angiography which was described in Chapter 2 overcomes several of these limitations. It enables a direct and non-invasive examination of the ocular microvasculature, including reproducible and accurate quantification of vessel density in the optic disc and peripapillary area (311). In addition, automated image reprocessing with software now commercially available on some OCT-A devices allows for exclusion of larger retinal vessels and a focused assessment of capillaries in three dimensional vascular beds. Vessel density is defined as a percentage of the selected image area occupied by 'flowing' vessels. A lower vessel density indicates loss or constriction of blood vessels but the exact physiological nature of these changes cannot be elucidated from the image.

Multiple studies published in the last few years have demonstrated attenuation of the network of capillaries directly feeding the optic nerve in glaucomatous eyes (312). Moreover, vessel density and indices of blood flow in the optic disc and peripapillary area correlate strongly with functional (VF) and structural (RNFL) defects (154,311,313,314). It is suspected that these vascular changes may by early signs of glaucoma, although

the crucial question of whether they are the cause or the consequences of neural damage remains unanswered.

Blood flow to the retina and the optic nerve can be potentially influenced by OSA and its treatment. On one hand, there is a risk that CPAP could reduce ocular perfusion by elevating IOP. On the other hand CPAP may hypothetically improve the optic nerve function by ameliorating the negative cardiovascular consequences of OSA. Of particular interest is endothelial dysfunction which is thought to be an early determinant of future vascular damage. CPAP therapy leads to clinically relevant improvement of endothelial function assessed by flow-mediated vascular dilatation (109). Also, some studies found that CPAP lowers serum levels of endothelin-1, a potent vasoconstrictor, although these data are not unanimous (315). Impaired blood flow in the cerebral circulation, which in many aspects is similar to the ocular circulation, has been demonstrated in people with OSA and there is some evidence that CPAP improves cerebral blood flow and reverses attenuated cerebrovascular responses to hypoxia (316–318).

The development of OCT angiography gives a unique opportunity to study networks of capillaries in OSA. So far this has been done in two recent studies (187,188). Yu et al. assessed retinal microvasculature in 69 Asian patients with untreated OSA and found that vessel densities in the peripapillary and parafoveal areas decreased with increasing severity of OSA. The peripapillary retinal vessel density was negatively correlated with AHI and SpO2, after adjusting for several potential co-variates, including age, BMI and OPP. These findings were however not confirmed in the study by Moyal et al. who, using the same OCT-A device, examined 53 slightly older Caucasian patients with mild, moderate and severe OSA and 28 control subjects at low risk of OSA. They found no significant differences in the optic disc, peripapillary and macular vessels densities across the groups.

There are no studies which examined the potential changes in OCT-A derived microvascular parameters after CPAP therapy. Hypothetically, by improving endothelial function and reducing endothelin-1 level and sympathetic activity CPAP would lead to relative dilatation of the retinal microvessels which could be detected as increased vessel density on OCT-A images.

The PAIR 2 study described in Chapter 4 was dedicated to assessment of nocturnal IOP changes but it also created an opportunity to examine OSA patients with POAG and healthy eyes before and directly after CPAP therapy with a newly acquired OCT-A device. This Chapter will examine the potential alterations in the microvasculature of the optic disc and peripapillary area after 4-6 weeks of CPAP therapy.

## 7.2 Methods

*7.2.1 Study population and procedures*

Participants of the PAIR 2 study were examined with an OCT-A device (AngioVueHD, Optovue Inc., Fremont, CA; Version: 2017.1.0.151) described in Chapter 2. The device was transported from Hinchingbrooke Hospital for each study visit to facilitate on site examination. Also, it was anticipated that acquisition of the images shortly after the participants woke up in the morning at Visit 1 (before CPAP) and Visit 2 ( after CPAP) would increase the chance of detecting changes to the ocular microvasculature potentially induced by CPAP at night.

All vascular parameters were automatically calculated by the Avanti SDOCT software (version: 2017.1.0.151). Whole image ONH vessels density measurements were taken from en face images acquired using the instrument defined 4.5 mm x 4.5 mm field of view centred on the optic disc. The cross-sectional boundaries of the examined tissue slab were defined by the internal limiting membrane and the RNFL. Circumpapillary vessel density measurements were calculated from a software defined 750 μm wide elliptical annulus extending outward from the optic disc boundary. The optic disc vessel density was measured based on the area inside the optic nerve head boundary. For the purpose of the analysis the following angiographic parameters were extracted:

- whole image optic nerve head vessel density (wiVD)
- circumpapillary vessel density (cpVD)
- optic disc vessel density (odVD)

For each of these parameters the vessel density was calculated for all vessels and, after exclusion of large vessels, separately for capillaries (see Appendix 3 for example of the software output).

Up to three repeated images were obtained from un-dilated eyes and the best quality image was then selected for each eye. The image quality index was automatically generated by the software which is programmed to rate each image on the scale from 1 (worst quality) to 10 (best quality). The following parameters are taken into account: signal strength, image clarity, residual motion artefacts, segmentation and disc centring. As advised by the manufacturer, image qualities of 5 and 6 are considered borderline and may potentially affect the calculation of vessel density (see Appendix 23 for examples of a range of different quality images). For this reason only participants with image quality of 7 and above in both eyes were included in the analysis.

The OCT-A examination was part of the PAIR 2 study protocol approved by the East of England – Cambridge South Research Ethics Committee (REC number 16/EE/0074, Appendix 12).

*7.2.2 Statistical analysis*

Comparisons of clinical characteristics between the groups, with the exception of ophthalmic variables, was performed using independent-samples T-test or, if the data were not normally or approximately normally distributed, and in case of categorical variables, using the Mann-Whitney U test. For ophthalmic data analysis both eyes were included. Baseline ophthalmic characteristics were compared using mixed model ANOVA with right and left eye as a within-subject factors and the study groups (POAG vs Control) as between-subject factors. To examine differences in pre- and post-CPAP OCT-A derived variables a repeat measures ANOVA was build.

Continuous data are presented as mean (SD) or median (IQR) depending on the distribution. Categorical data are reported as percentages. Normality was checked using Shapiro-Wilk test and by assessment of skewness and its standard error.

The level of significance for each comparison was set at $p < 0.05$. All analyses were conducted using SPSS software version 22.0 (IBM SPSS, IL, USA).

## 7.3 Results

Of the 26 patients who took part in the PAIR 2 study, 13 participants including: 7 patients with POAG (14 eyes) and 6 control subjects (12 eyes) were included in the analysis. The OCT-A scans could not be obtained during one study visit due to technical problems with the device. This resulted in a loss of angiographic data on 2 patients. The remaining 11 cases were excluded based on a suboptimal OCT-A image quality in at least one eye pre- or post-CPAP therapy. Demographic and clinical characteristics of participants are summarised in Table 7-1.

All OCT-A indices in eyes of POAG patients were significantly lower than in control subjects. The differences remained strongly significant after adjusting for age (Table 7-1).

However, in neither group were there any significant changes in the optic disc, peripapillary area and the whole image vessel densities after CPAP therapy (Table 7-2).

Table 7-1. Baseline characteristics of participants included in the study (T-test or Mann-Whitney U test or mixed model ANOVA).

| | POAG N=7 (14 eyes) | Controls N=6 (12 eyes) | P value |
|---|---|---|---|
| Age (yr), sd | 67±5.6 | 59±10.3 | 0.082 |
| Sex (% Male) | 43 | 67 | 0.41 |
| BMI (kg/m2), sd | 33.9±5.4 | 35±5.8 | 0.74 |
| AHI (per h), sd | 30.6±13.1 | 36.7±19.6 | 0.52 |
| $SBP_{upright}$ (mmHg), sd | 149±11.5 | 138±26 | 0.33 |
| $DBP_{upright}$(mmHg), sd | 85±14.5 | 84±8.3 | 0.86 |
| Hypertension,% | 43 | 50 | 0.81 |
| CPAP level (cmH2O), sd | 10.1±2.2 | 11.5±2 | 0.24 |
| CPAP usage (h/nt), sd | 5.3±1.7 | 4.8±1.9 | 0.62 |
| Residual ODI (per h), IQR | 6.8±2.2 | 2.8±9.4 | 0.2 |
| $\Delta OPP_{(Visit2-Visit1)}$, mmHg, sd | 1.4±3.6 | -7.1±6.2 | 0.011 |
| VA (LogMAR), sd | 0.041±0.12 | 0.017±0.12 | 0.72 |
| MD (dB), sd | -2.5±1.3 | -1.1±1.3 | 0.1 |
| cRNFL (µm), sd | 86.7±7.6 | 111.4±7.6 | 0.000 |
| OCT-A Vessel Density | | | |
| $wiVD_A$ (%), sd | 49.7±2.7 | 55.5±2.7 | $0.003^{\infty}$ |
| $wiVD_C$ (%), sd | 43.2±2.7 | 49.0±2.7 | $0.003^{®}$ |
| $idVD_A$ (%), sd | 56.1±2.9 | 62.1±2.9 | $0.003^{*}$ |
| $idVD_C$ (%), sd | 48.8±3.2 | 54±3.2 | $0.015^{\#}$ |
| $cpVD_A$ (%), sd | 51.5±2.6 | 57.7±2.7 | $0.002^{\&}$ |
| $cpVD_C$ (%), sd | 44.9±3.2 | 51.6±3.2 | $0.003^{\dagger}$ |

Abbreviations: BMI=body mass index, SBP=systolic blood pressure, DBP=diastolic blood pressure, AHI=apnoea-hypopnoea index, CPAP=continuous positive airway pressure, Residual ODI=oxygen desaturation index measures on CPAP at Visit 2, VA=visual acuity, MD=mean deviation, cRNFL=global circumpapillary retinal nerve fibre layer thickness, ΔOPP=change in calculated nocturnal ocular perfusion pressure between visit 2 and visit 1, OCT-A=ocular coherence tomography angiography, $VD_A$=vessel density for all vessels, $VD_C$=vessel density for capillaries, wi=whole image area, id=inside the optic disc, cp=circumpapillary.

After adjusting for age the following p values were obtained respectively: $^{\infty}$0.008, $^{®}$0.006 $^{*}$0.012, $^{\#}$0.031, $^{\&}$0.004, $^{\dagger}$0.005.

Table 7-2. Comparison of the optic nerve vessel density (OCT-A indices) before and after CPAP therapy (repeated measures ANOVA).

| | Before CPAP | After CPAP | P value |
|---|---|---|---|
| POAG group, N=7 (14 eyes) | | | |
| $wiVD_A$ (%), sd | 49.7±2.9 | 49.4±2 | 0.7 |
| $wiVD_C$ (%), sd | 43.2±3.1 | 43.1±2.6 | 0.89 |
| $idVD_A$ (%), sd | 56.1±3.4 | 55.6±5.3 | 0.77 |
| $idVD_C$ (%), sd | 48.8±4.1 | 48.6±5.6 | 0.89 |
| $cVD_A$ (%), sd | 51.5±3.1 | 51.5±3 | 0.97 |
| Superior hemifield, sd | 52.2±3.2 | 52.1±3.2 | 0.93 |
| Inferior hemifield, sd | 50.6±3.4 | 50.8±3 | 0.82 |
| $cVD_C$ (%), sd | 44.9±3.9 | 45.1±4.23 | 0.73 |
| Superior hemifield, sd | 45.3±4.2 | 45±.4.8 | 0.88 |
| Inferior hemifield, sd | 44.3±4 | 44.6±4 | 0.67 |
| Control group, N=6 (12 eyes) | | | |
| $wiVD_A$ (%), sd | 55.5±2.4 | 55.8±2.8 | 0.6 |
| $wiVD_C$ (%), sd | 49±2.1 | 49.3±2.42 | 0.56 |
| $idVD_A$ (%), sd | 62.1±1.8 | 61.9±3.9 | 0.86 |
| $idVD_C$ (%), sd | 54±1.57 | 54.1±3.7 | 0.97 |
| $cVD_A$ (%), sd | 57.7±2.1 | 58.1±2.3 | 0.61 |
| Superior hemifield, sd | 58.9±2.3 | 59.4±2.7 | 0.56 |
| Inferior hemifield, sd | 56.4±2.4 | 56.6±2.9 | 0.69 |
| $cVD_C$ (%), sd | 51.6±1.9 | 51.9±2.16 | 0.59 |
| Superior hemifield, sd | 52.7±2.1 | 53±2.8 | 0.65 |
| Inferior hemifield, sd | 50.3±2.4 | 50.7±2.7 | 0.57 |

Abbreviations: BMI=body mass index, CPAP=continuous positive airway pressure, OCT-A=ocular coherence tomography angiography, $VD_A$=vessel density for all vessels, $VD_C$=vessel density for capillaries, wi=whole image area, id=inside the optic disc, cp=circumpapillary.

### 7.4 Discussion

The development of OCT-A opens new opportunities not only to examine the role of retinal microvasculature in glaucoma and other ocular conditions but may also provide new insights into the systemic cardiovascular impact of diseases such as OSA or essential hypertension. However, this is a new technology and much is still to be learnt about its advantages, limitations and, importantly, the underlying physiological mechanisms which drive the alterations in OCT-A defined vessel density and blood flow. It is yet to be established whether these vascular measurements have sufficient dynamic range to allow detection of clinically significant changes in response to an intervention.

Undoubtedly, more studies comparing ocular microvasculature in people with and without OSA well matched for variables which have been shown or may be potentially associated with the angiographic parameters, are clearly needed.

This study confirms that in POAG patients, even in those with mild visual field impairment, there is a significant drop out of retinal capillaries when compared with the control group. However, no changes in the angiographic parameters were observed after CPAP therapy in either group. Despite between-group differences in CPAP related change in calculated OPP the microvascular indices remained unaltered in both groups. These results provide some reassurance that nocturnal IOP elevation caused by CPAP is not high enough to compromise retinal microvasculature, at least when it is measured shortly after the sleep period. However, it is also possible that OCT-A may not be sensitive enough to detect the impact of smaller IOP changes which may compromise ocular perfusion in addition to causing mechanical stress to the optic nerve. Indeed, a relationship between IOP and angioflow indices measured by OCT-A was demonstrated in a recent proof-of-concept study but a reduction of IOP by at least 50% from baseline values of IOP≥35 mmHg in patients with newly diagnosed HTG and ocular hypertension was needed to observe potentially clinically meaningful increase of peripapillary capillary perfusion in the RNFL (319). Similarly, another study also reported reduction of the area of vascular drop out in the peripapillary microvasculature associated with reduction of IOP from 26 to 13mmHg three months after trabeculectomy (320).

The current study raises doubts on whether CPAP therapy could improve blood supply to the optic nerve in the age range when glaucoma is typically diagnosed. Age has been identified as a factor in OCT-A measured retinal vessel density; older patients have lower macular and peripapillary vessels densities than younger counterparts (321). Age, as well as cardiovascular co-morbidities, may also be factors in reversibility of the potential OSA related changes in the ocular microvasculature. The association between OSA and hypertension, cardiovascular diseases and mortality is much stronger in younger people (322,323). Thus, it could be hypothesised that with increasing age the likelihood of

reversing cardiovascular changes with CPAP decreases even if they are contributed by OSA. Interestingly, in a recent study in paediatric patients an improvement in some microvascular retinal indices measured with OCT-A was reported one month after adenoidectomy, although that study had some important weaknesses, including the lack of objective confirmation of OSA with a sleep test in operated children (324).

There are two main limitations of the current opportunistic study. Firstly, the sample size was small and may have been insufficient to demonstrate significant differences in the paired OCT-A measurements. As complete angiographic data suitable for analysis (two eyes, two repeated measurements) were available only for 50% of all subjects I cannot exclude selection bias. The main reason for exclusion was suboptimal image quality. In total, 96 scans were obtained on 24 subjects. Of these, 29.8% images were low quality. This is in keeping with the proportion of poor quality scans excluded in other studies (321). One specific challenge for obtaining high quality OCT-A images in the current study was corneal dryness from repeated IOP measurements at night which affected some patients and which was not always immediately helped by application of lubricants. Also, as most of our participants wished to drive back home straight after completing all study assessments, we could not use mydriatic eye drops. It is therefore possible that if the scans were taken in different circumstances the proportion of rejected images would have been lower.

Secondly, the CPAP treatment period was relatively short which would allow detection of only early vascular changes. Whilst some studies found significant improvement in endothelial function after only 4 weeks of CPAP therapy a longer period of treatment may be required for the microvascular changes to be demonstrated with OCT-A (325). In addition, it is not yet established whether patients with OSA have reduced retinal vessel density. Since, unlike for structural RNFL measurements, there are no reference values available yet, I could not determine if the baseline OCT-A indices in the control group were lower than what would be expected for a population based sample of the corresponding age.

Because of these limitations firm conclusions cannot be drawn from this pilot study. However, these data could be helpful in designing future studies which should consider allowing longer period of CPAP therapy and potentially recruiting younger patients with OSA and without established cardiovascular risk factors. It would be also interesting to examine how OCT-A indices relate to the established markers of endothelial dysfunction such as flow-mediated vasodilatation.

### 7.5 Conclusions

In this pilot study there were no significant changes in the indices of the optic nerve microvasculature assessed with OCT-A after 4-6 weeks of CPAP therapy in people with and without glaucoma. Longer duration and larger studies are needed to determine whether a long-term use of CPAP affects the vascular parameters potentially relevant to the progression of the optic neuropathy.

# Chapter 8: Conclusions

OSA has been proposed to be linked with POAG for over two decades but it is still unclear how relevant it is to the development and progression of glaucomatous neuropathy. Given the irreversible nature of vision loss in glaucoma and limited treatment options, particularly for those patients who have progressive disease despite adequate IOP lowering, it is important that the relevance of this common sleep disorder is established.

Despite the routine practice of treating patients with OSA and concomitant glaucoma with CPAP there is a lack of evidence on what effect, if any, CPAP has on their IOP. These gaps in our knowledge prevents clinicians from giving appropriate advice to patients with glaucoma commencing CPAP therapy about the possible side-effects and potential ways of mitigating them.

The studies described in this thesis clarify some of these pertinent issues and provide better quality evidence in several areas but also raise more questions which could set directions for future research.

Below, I summarise the main findings of the five thesis chapters which reported the results of original studies and the conclusions drawn from them. I then summarise the novel contributions of the thesis and describe further questions arising from this research.

## 8.1 Summary

The study described in Chapter 3 investigated the prevalence of OSA among unselected POAG patients recruited from glaucoma clinics in a secondary care hospital in the United Kingdom. The prevalence of OSA was found to be comparable to that reported in the general population of middle to elderly age people and no different to statistically matched control subjects. Importantly, the burden of OSA including its traditional severity markers and the associated sleepiness did not differ between POAG patients and controls. Thus, it was concluded that if routine OSA screening was implemented in glaucoma clinics it would likely yield a similar proportion of patients who would then be considered for OSA treatment as screening conducted in the general population of a similar age range. Moreover, it was demonstrated that the STOP-BANG questionnaire which had been proposed for OSA screening in glaucoma patients, has no useful discriminative value and should not be used in this disease-specific cohort of patients. Although it has been suspected that OSA may be more relevant in NTG than in HTG, both subgroups of POAG had the same prevalence and severity of OSA. I have found no correlation between OSA severity and markers of functional and structural glaucomatous damage in the cross-sectional analysis.

The study reported in Chapter 4 retrospectively assessed rates of structural POAG progression in people with and without OSA. The key finding was that patients with untreated OSA had significantly faster rates of RNFL loss independently of other examined systemic and ocular factors previously associated with glaucoma progression. It was argued that the observed differences in RNFL thinning may be clinically relevant although this would require confirmation in future prospective studies with concomitant monitoring for structural and functional progression of glaucomatous damage.

The study in Chapter 5 sought to determine whether CPAP therapy increases IOP at night in people with and without glaucoma. This was examined by repeated IOP measurements performed during two separate visits before and during CPAP application. The findings of two previous studies which demonstrated a significant CPAP-induced IOP elevation at night in people without glaucoma were confirmed. Importantly, for the first time, the same effect of CPAP has been demonstrated in people with POAG in whom IOP elevation was not prevented by ocular hypotensive therapy. A moderate correlation between CPAP level and the magnitude of IOP increase observed in this study indicates that it would be reasonable to avoid high CPAP level for patients with glaucoma although IOP increase is unlikely to be explained by the level of CPAP alone.

The experiment described in Chapter 6 was designed to examine the hypothesis of pressure transmission as the predominant mechanisms of IOP elevation during the CPAP exposure. It was assumed that if this hypothesis was correct a correlation between IOP and incremental levels of CPAP would be detected during a daytime CPAP application. However, no significant change in IOP was observed in awake subjects with or without glaucoma, including untreated glaucoma, despite CPAP application for up to 2 hours and in the pressure range most commonly used in clinical practice. It was concluded that whilst positive pressure transmission may still occur at night other mechanisms could play a part in CPAP related IOP increases and that sleep is essential to observe the relationship between CPAP and IOP.

Chapter 7 described a pilot study which explored whether a short course of CPAP to treat OSA in people with and without glaucoma induces changes in the optic nerve microvasculature examined with a novel angiographic imaging technique (OCT-A). The study found that the vascular parameters potentially relevant to the progression of the optic neuropathy remained unaltered after 4-6 weeks of CPAP therapy.

## 8.2 Thesis contributions

This thesis has contributed to the field in multiple ways:

- The thesis has produced the largest to date prospective study with detailed ocular and sleep apnoea assessment which examined the prevalence of OSA among glaucoma patients in a 'real-world' clinic scenario and had a concurrent control group. Through this, the thesis has provided estimates of OSA frequency and severity among unselected POAG patients and contributed to the debate on the role of systematic screening for OSA in glaucoma clinics.
- The thesis has established that the STOP-BANG questionnaire is not a useful screening tool in POAG patients.
- The thesis has found that the burden of OSA is not different in patients with NTG and HTG and that there is likely no need to separate out these two subgroups of POAG when examining the effects of OSA in future studies.
- The thesis has established that there is no cross-sectional association between OSA severity and the severity of POAG measured by functional and structural indices.
- The thesis has found that among POAG patients the rate of RNFL loss is significantly faster in people diagnosed with OSA than in those without OSA and that OSA may be an independent risk factor for structural progression of POAG.
- The thesis has confirmed that CPAP raises IOP at night in people with healthy eyes and provided new evidence that this treatment also causes statistically and potentially clinically significant elevation of nocturnal IOP in people with treated POAG and that IOP elevation is partly dependent on the CPAP level.
- The thesis has refuted the previous suggestion that the application of CPAP causes an immediate IOP increase in awake subjects which weakens the previously proposed hypothesis of mechanical pressure transmission as the predominant mechanism of CPAP related IOP elevation.
- The thesis has assessed ocular microvasculature using OCT-A in OSA patients with and without POAG and in a pilot study found no indication that CPAP alters these microvascular parameters in the short-term.

### 8.3 Final conclusions and future directions

The relationship between OSA and POAG is complex and multilevel. Future, well-designed studies are needed to unravel various aspects of it and provide good quality evidence to guide clinical practice.

This thesis has not directly examined whether OSA is an important risk factor for the development of POAG but the observation that people with POAG are not more likely to be diagnosed with OSA and that the severity of it is not associated with POAG severity would argue that OSA is not potent enough to alone cause glaucomatous damage. It may however be an important progression risk factor in people who have already developed POAG as a result of unfavourable balance between IOP and the optic nerve head pressure sensitivity or other risk factors. This is one of the most important directions for future research. Prospective longitudinal studies with concomitant monitoring for structural and functional progression whilst accounting for other risk factors and perhaps with repeated assessment for OSA severity, could provide essential data and are the natural way forward before interventional studies are considered. This thesis provides data to help with design of such studies, not only in terms of the sample size calculation but also in terms of ethical considerations. It was demonstrated that the majority of POAG patients diagnosed with OSA do not have relevant symptoms which would require treatment with CPAP based on the current practice, and thus a longer monitoring period without an intervention which could potentially affect glaucoma progression outcomes, would be ethically justifiable.

Equally important is to establish whether CPAP is a safe treatment modality for patients with POAG. The demographics of POAG and OSA overlap in that sense that POAG is typically diagnosed after the age of 50 years and OSA prevalence peaks at the age of around 65 years. As both conditions are common, despite the lack of cross-sectional association between them we are likely to encounter POAG patients who require CPAP therapy for coexisting OSA. The IOP raising effect of CPAP is likely to go unnoticed as it does not carry over to wakefulness and IOP is typically only monitored during the daytime office hours in glaucoma clinics. Given this, and the slowly progressive nature of glaucomatous neuropathy nocturnal IOP may remain elevated for a long before CPAP is considered as a potential contributor, if indeed the treating clinicians ever developed this hypothesis. This thesis demonstrated that CPAP raises nocturnal IOP and this is something that both sleep specialist and ophthalmologists as well as patients should be aware of. However, it does not mean that CPAP would necessarily have a negative impact on important glaucoma outcomes. In fact, the reverse may be true. Currently, there is no sufficient ground to recommend changes in practice. The long-term effect of

CPAP on glaucomatous neuropathy can only be established in RCTs with well-matched groups and this would be another direction for future research. Further physiological studies dedicated to a better understanding of mechanisms behind CPAP induced IOP elevation could inform the design of such interventional studies and guide clinicians in what steps could be taken to mitigate the possible adverse effects of CPAP therapy.

Also, on the physiological level there is a need to understand which of the deleterious systemic effects of OSA are most relevant to RGCs degeneration. Although CPAP can reverse endothelial dysfunction the results of RCTs dedicated to examining the role of CPAP in preventing important cardiovascular outcomes have been mostly and disappointingly negative. Demonstrating cardiovascular or cerebrovascular benefits of CPAP may be even more challenging in the middle to elderly age groups. If, however, OSA affects RGCs directly by augmenting oxidative stress or worsening hypoxia in the cells particularly sensitive to it, CPAP may stand a better chance of making a positive difference. Establishing whether there is a relationship between intermittent hypoxia and oxidative stress in the ocular tissues in POAG patients would inform this debate.

Beyond glaucoma, the OCT measurements provide easily accessible window to the central nervous system and the RNFL could be a useful biomarker in OSA research on neurodegeneration. According to a recent population based study conducted in the UK a thinner RNFL is associated with worse cognitive function also in individuals without a neurodegenerative disease and carries a greater likelihood of future cognitive decline (326). Thus retinal degeneration has been proposed as a biomarker of preclinical dementia (327). RNFL assessment could aid risk stratification and potentially help to identify those OSA patients who are at a higher risk of future cognitive dysfunction. It could also be an intermediate outcome measure in interventional studies examining the role of CPAP in reducing the risk of dementia. Studies dedicated to assessment of longitudinal RNFL changes in OSA patients with healthy eyes are yet to be performed.

In summary, this thesis found no epidemiological links between OSA and POAG but has provided data in support of the role of OSA in the progression of glaucomatous neuropathy in people with already established POAG. In addition, it has been demonstrated that CPAP raises IOP also in people with treated POAG. Future studies should focus on examining the long-term effects of OSA and CPAP therapy on important glaucoma outcomes.

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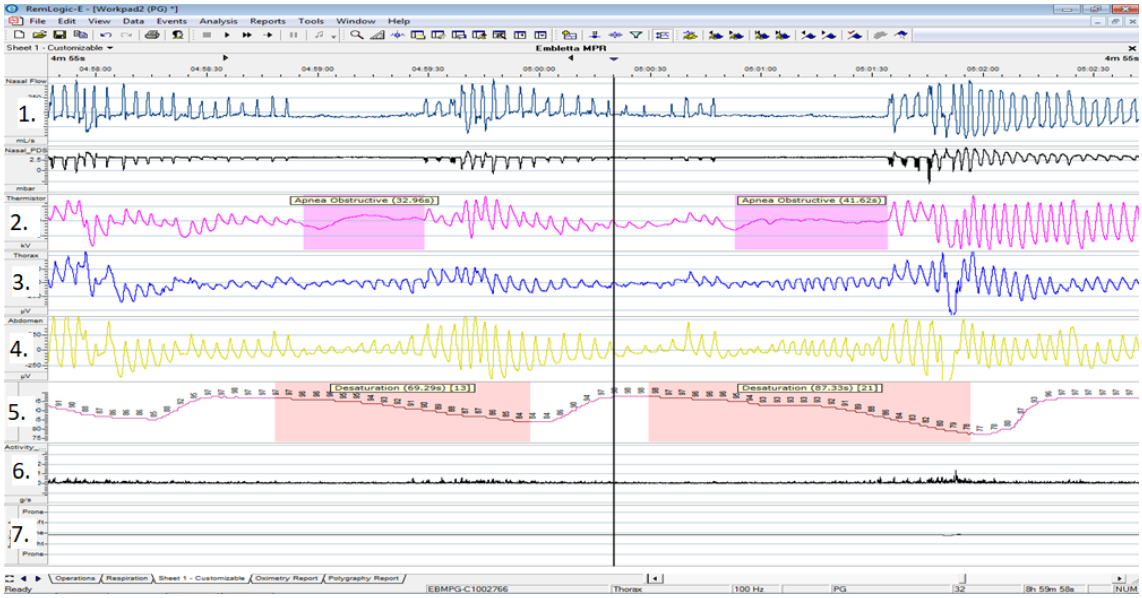
325. Ip MSM, Tse H-F, Lam B, Tsang KWT, Lam W-K. Endothelial function in obstructive sleep apnea and response to treatment. Am J Respir Crit Care Med. 2004 Feb 1;169(3):348–53.

326. Ko F, Muthy ZA, Gallacher J, Sudlow C, Rees G, Yang Q, et al. Association of Retinal Nerve Fiber Layer Thinning With Current and Future Cognitive Decline: A Study Using Optical Coherence Tomography. JAMA Neurol. 2018 Oct 1;75(10):1198–205.

327. Fyfe I. Retinal nerve fibre layer thickness - a biomarker of early dementia? Nat Rev Neurol. 2018 Aug;14(8):449.

# List of Appendices

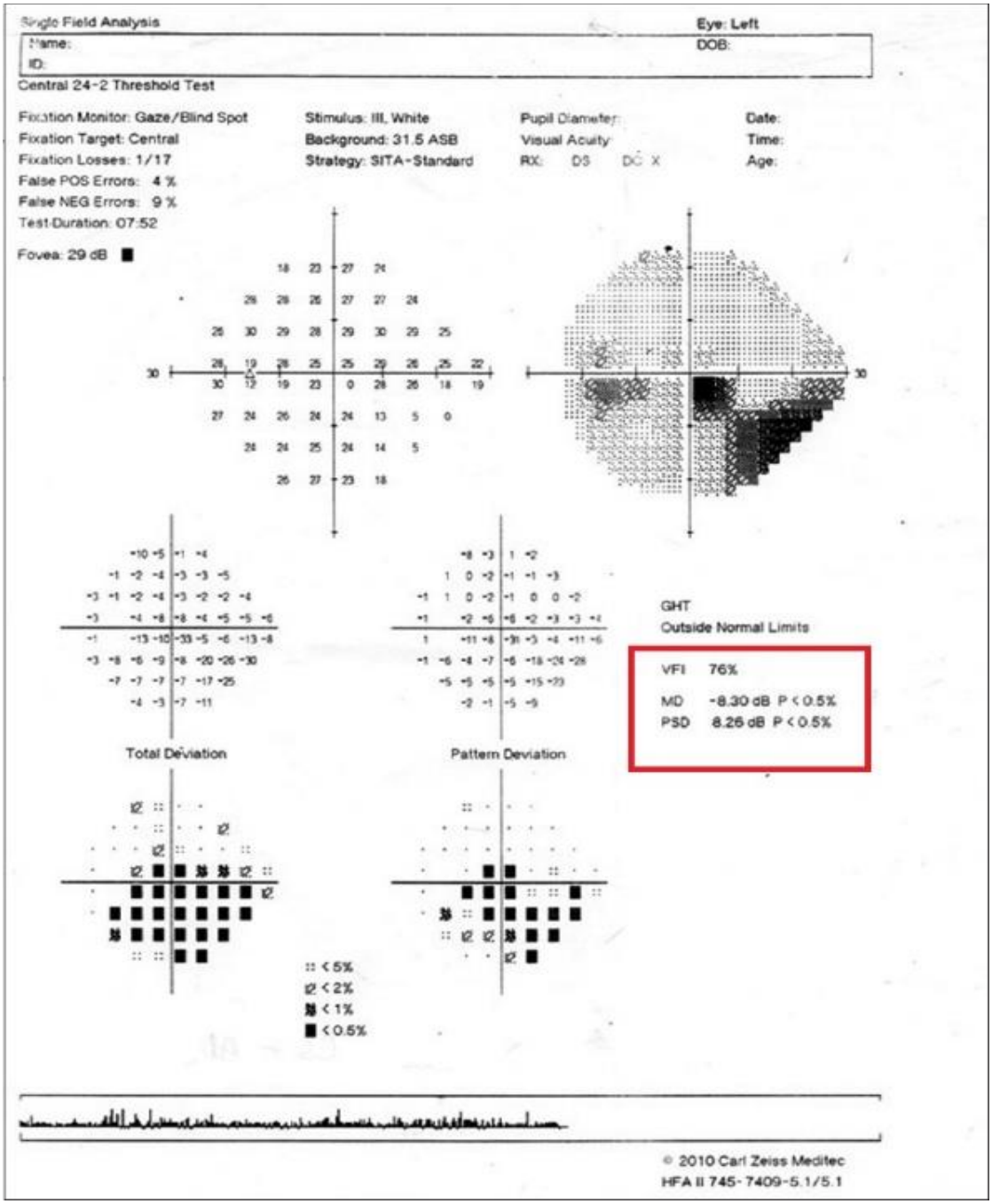
Appendix 1. Example of rPG recording with manually scored respiratory events in a patient with POAG who was found to have moderate OSA.




The screenshot represents just under 5 minutes of recording with two long obstructive apnoeas leading to oxygen desaturation to 84% and 77%, respectively.

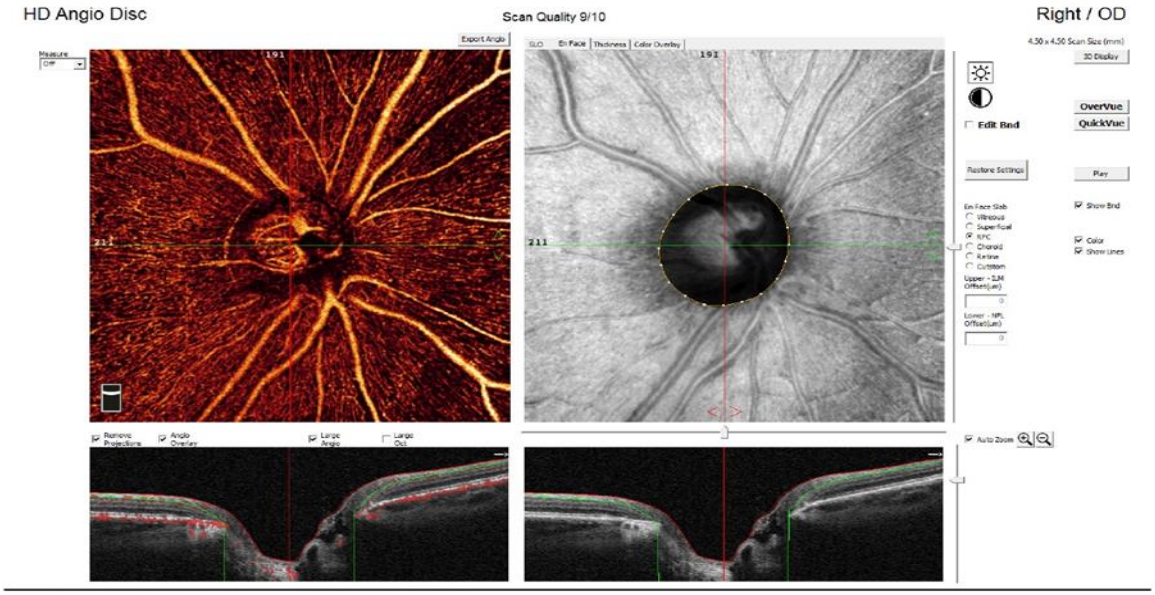
Description of signals: 1. Nasal flow (used for scoring hypopnoeas), 2. Thermistor signal (used for scoring apnoeas), 3. Chest respiratory movements, 4. Abdominal respiratory movements, 5. Oximetry, 6. Activity signal, 7. Body position signal.

Appendix 2. Example of HFA output in a patient with glaucomatous visual field defect.

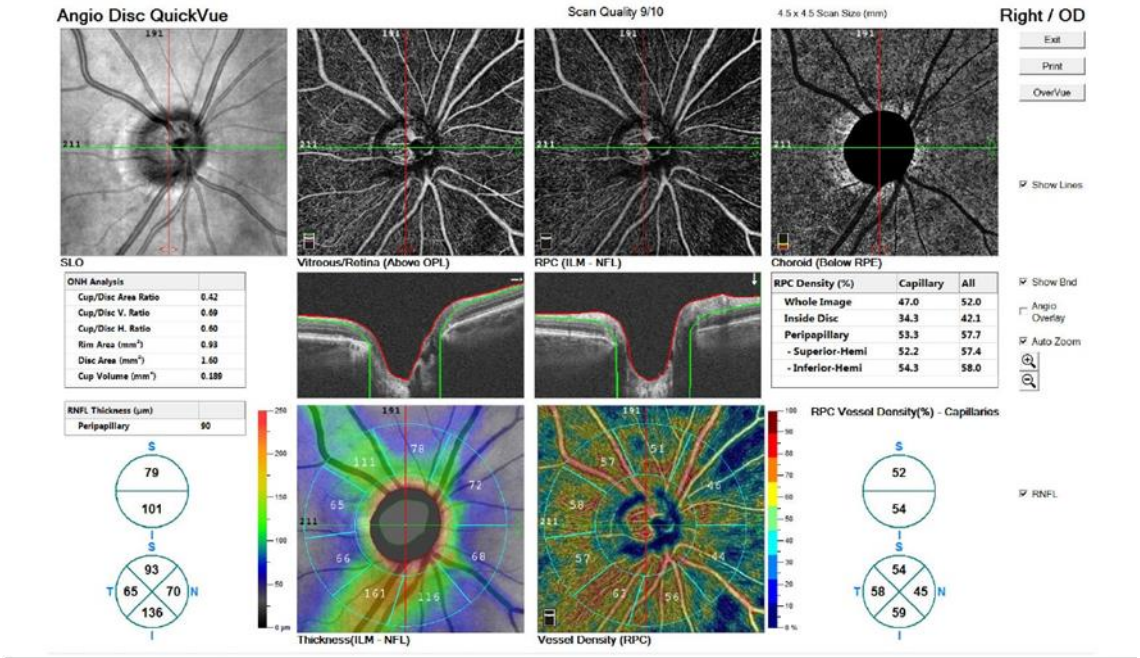


Appendix 3. Example of OCT-A images acquired on a POAG patient with mild visual field defect.

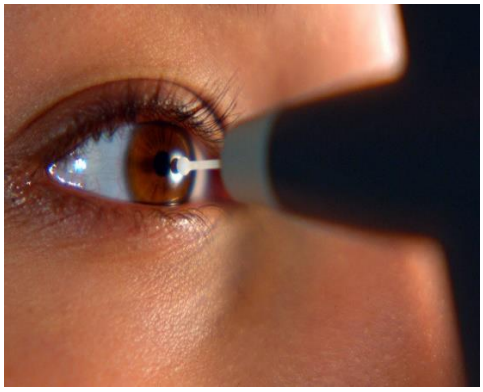
A. “Angio disc” images demonstrating automated optic disc boundary determination based on scanning laser ophthalmoscopy (SLO) and radial peripapillary capillaries (RCP) layer determination on cross sectional images.




B. En face OCT-A images of three layers of the disk and peripapillary area (the vitreous level, the radial peripapillary capillaries layers-RCP, and the superficial choroid) with software output for vessels densities in the RCP layer.



Appendix 4. IcarePro (top pictures)  and IcareHome (bottom pictures) tonometers.




Appendix 5. Ethical approvals for the POSAG study (Chapters 3 and 4)

NHS
Health Research Authority

**East of England - Cambridgeshire and Hertfordshire Research Ethics Committee**
Royal Standard Place
Nottingham
NG1 6FS

Telephone: 0115 8839435
Fax: 0115 8839294

24 September 2015

Dr Ian Smith
Consultant Respiratory and Sleep Physician
Papworth Hospital NHS Trust
Respiratory Support & Sleep Centre
Papworth Hospital
Papworth Everard, Cambridge
CB23 3RE

Dear Dr Smith

| | |
|---|---|
| **Study title:** | **POSAG Trial: Prevalence of Obstructive Sleep Apnoea in GlaucomaProspective observational controlled trial of the prevalence of obstructive sleep apnoea in patients with open-angle glaucoma.** |
| **REC reference:** | **15/EE/0292** |
| **IRAS project ID:** | **183820** |

Thank you for your letter 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 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 Manager, Miss Georgia Copeland, nrescommittee.eastofengland-cambsandherts@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 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*

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" below).

Non-NHS sites

**Approved documents**

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

| *Document* | *Version* | *Date* |
|---|---|---|
| Covering letter on headed paper [Response to provisional REC opinion] | | 03 September 2015 |
| GP/consultant information sheets or letters | | |
| GP/consultant information sheets or letters | 1 | 03 July 2015 |
| IRAS Checklist XML [Checklist_11092015] | | 11 September 2015 |
| Letters of invitation to participant | 2 | 03 September 2015 |
| Non-validated questionnaire | | |
| Non-validated questionnaire | | |
| Non-validated questionnaire | | |
| Non-validated questionnaire | | |
| Other [Participant Screening Form Glaucoma Patient] | | |
| Other [Screening Form Control Subjects I] | | |
| Other [Screening Form Control Participants II] | | |
| Participant consent form | 2 | 03 September 2015 |
| Participant information sheet (PIS) [Glaucoma patients] | 2 | 03 September 2015 |
| Participant information sheet (PIS) [Control Participants I-attending clinic] | 2 | 03 September 2015 |
| Participant information sheet (PIS) [Control Participants II-not attending clinic] | 2 | 03 September 2015 |
| REC Application Form [REC_Form_11092015] | | 11 September 2015 |
| Research protocol or project proposal | | |
| Research protocol or project proposal | 5 | 03 September 2015 |
| Summary CV for Chief Investigator (CI) | | |
| Summary CV for student | | |
| Summary CV for supervisor (student research) | | |
| Validated questionnaire | | |
| Validated questionnaire | | |

**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/

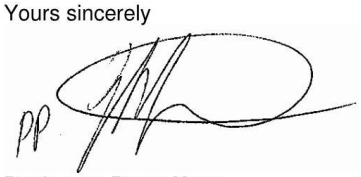
**HRA Training**

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/EE/0292** | **Please quote this number on all correspondence** |
|---|---|

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

Yours sincerely



**Professor Barry Hunt**
**Chair**

Email:nrescommittee.eastofengland-cambsandherts@nhs.net

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

*Copy to:* *Dr Victoria Stoneman , Research & Development*

Anglia Ruskin University

Cambridge Chelmsford Peterborough

**Ref**: NS/jc/FMSFREP/15/16 014
**Enquiries**: Joanne Corney
**Direct Line**: 01245 684779
**Date**: 29th October 2015

**Chelmsford Campus**
Bishop Hall Lane
Chelmsford
CM1 1SQ

T: 0845 196 4779
Int: +44 (0)1245 493131
www.anglia.ac.uk

Dariusz Wozniak

Dear Dariusz

**Re: Application for Ethical Approval**

| | |
|---|---|
| **Principal Investigator:** | Dariusz Wozniak |
| **FREP number:** | 15/16 014 |
| **Project Title:** | POSAG Trial: Prevalence of Obstructive Sleep Apnoea in Glaucoma. Prospective observational controlled trial of the prevalence of obstructive sleep apnoea in patients with open-angle glaucoma. |

Thank you for your application for ethical approval which has now been considered by the Faculty (of Medical Science) Research Ethics Panel (FREP).

I am pleased to inform you that your application has been approved by the Faculty Research Ethics Panel Chair under the terms of Anglia Ruskin University's Research Ethics Policy (Dated 23/6/14, Version 1).

Ethical approval is given for a period of 3 years from Thursday 29th October 2015.

It is your responsibility to ensure that you comply with the Research Ethics Policy and Code of Practice for Applying for Ethical Approval at Anglia Ruskin University and specifically:

- The procedure for submitting substantial amendments to the committee, should there be any changes to your research. You cannot implement these changes until you have received approval from FREP for them.
- The procedure for reporting adverse events and incidents.
- The Data Protection Act (1998) and any other legislation relevant to your research. You must also ensure that you are aware of any emerging legislation relating to your research and make any changes to your study (which you will need to obtain ethical approval for) to comply with this.
- Obtaining any further ethical approval required from the organisation or country (if not carrying out research in the UK) where you will be carrying the research out. Please ensure that you send the FREP copies of this documentation if required, prior to starting your research.

- Any laws of the country where you are carrying the research and obtaining any other approvals or permissions that are required.
- Any professional codes of conduct relating to research or research or requirements from your funding body (please note that for externally funded research, a Project Risk Assessment must have been carried out prior to starting the research).
- Completing a Risk Assessment (Health and Safety) if required and updating this annually or if any aspects of your study change which affect this.
- Notifying the FREP Secretary when your study has ended.

Information about the above can be obtained on our website at:

http://web.anglia.ac.uk/anet/rdcs/ethics/index.phtml

Please also note that your research may be subject to random monitoring by the Panel.

Should you have any queries, please do not hesitate to contact my office. May I wish you the best of luck with your research.

Yours sincerely,

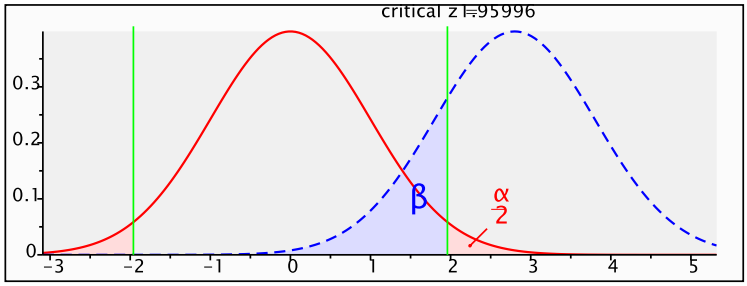
**Dr Nigel Sansom**
**Director of Research**
For the Faculty (of Medical Science) Research Ethics Panel

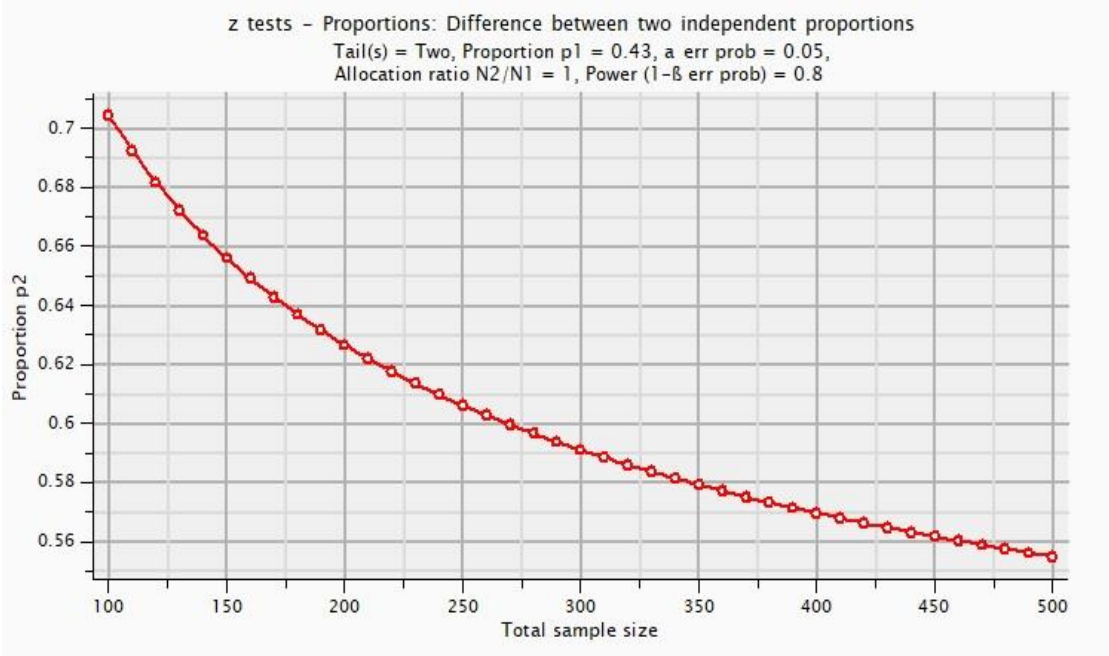
Appendix 6. Sample size calculation for the POSAG study and a plot demonstrating the required sample size as a function of OSA prevalence in the POAG group (G*Power output)

*- Saturday , November 21, 2015 -- 18:54:51*

**z tests -** Proportions: Difference between two independent proportions

| | | | |
|---|---|---|---|
| **Analysis:** | A priori: Compute required sample size | | |
| **Input:** | Tail(s) | = | Two |
| | Proportion p2 | = | 0.56 |
| | Proportion p1 | = | 0.43 |
| | α err prob | = | 0.05 |
| | Power (1−β err prob) | = | 0.8 |
| | Allocation ratio N2/N1 | = | 1 |
| **Output:** | Critical z | = | 1.959964 |
| | Sample size group 1 | = | 232 |
| | Sample size group 2 | = | 232 |
| | Total sample size | = | 464 |
| | Actual power | = | 0.801686 |






Appendix 7. Baseline demographic characteristics of invited POAG patients.

| | POAG patients who took part in the study (n=251) | POAG patients who declined participation in the study (n=307) | P value |
|---|---|---|---|
| Age, yr, (sd) | 70.8 (10) | 77.4 (8.9) | 0.000 |
| Male sex, % | 56.5 | 46.9 | 0.025 |

Appendix 8. Predictors of OSA (AHI≥5) in the entire POSAG study cohort based on univariate logistic regression analyses.

| Predictor | Nagelkerke R2 | OR | 95% CI | P value |
|---|---|---|---|---|
| Age | 0.055 | 1.05 | 1.023-1.071 | 0.000 |
| Male sex | 0.021 | 1.67 | 1.1-2.5 | 0.013 |
| BMI | 0.12 | 1.16 | 1.1-1.23 | 0.000 |
| Neck size | 0.09 | 1.16 | 1.09-1.2 | 0.000 |
| Diabetes | 0.018 | 2.25 | 1.1-4.6 | 0.028 |

Definition of abbreviations: OSA=obstructive sleep apnoea, AHI-apnoea-hypopnoea index, BMI=body mass index.

Appendix 9. Comparison of POAG patients with more severe visual field defect (eGSS stages 2-5) with the control group in relation to OSA.

| | POAG patients (n=177) | Controls (n=160) | P value |
|---|---|---|---|
| OSA, n (%) | 101 (57.1) | 87 (54.4) | 0.66 |
| Moderate to severe OSA, n (%) | 38 (21.5) | 26 (16.3) | 0.27 |
| AHI, per h, (IQR) | 5.9 (11) | 5.7 (9.7) | 0.48 |
| ODI, per h, (IQR) | 3.8 (7.1) | 3.3 (7.1) | 0.63 |
| T90, %, (IQR) | 14 (78) | 15 (76) | 0.77 |
| MeanSpO2, %, (IQR) | 93.1 (1.9) | 93 (2.0) | 0.84 |
| MinSpO2, %, (IQR) | 86 (6) | 86 (6.0) | 0.92 |
| ESS score, (IQR) | 6 (6) | 5 (6.0) | 0.92 |

Definition of abbreviations: POAG=primary open-angle glaucoma, eGSS=enhanced Glaucoma Staging System, OSA=obstructive sleep apnoea, AHI=apnoea-hypopnoea index, ODI=oxygen desaturation index, T90=time spent with oxygen saturation less than 90%, MeanSpO2=mean oxygen saturation, MinSpO2=minimum oxygen saturation, ESS=Epworth Sleepiness Scale.

Appendix 10. HTG vs NTG-Baseline Characteristics of Participants.

| | HTG subgroup (n=139) | NTG subgroup (n=77) | P value |
|---|---|---|---|
| *Baseline characteristic of participants in relation to OSA* | | | |
| Male sex, n (%) | 89 (64) | 34 (44.2) | 0.006 |
| Age, yr, (sd) | 69.8 (9.3) | 71 (9.4) | 0.375 |
| White European, n (%) | 137 (98.6) | 72 (93.5) | 0.14 |
| BMI, kg/m$^2$, (sd) | 27.9 (4.3) | 26.6 (4.3) | 0.041 |
| Diabetes type 2, n (%) | 15 (10.8) | 10 (13) | 0.66 |
| Hypertension, n (%) | 64 (46) | 36 (46.8) | 1.00 |
| IHD, n (%) | 14 (10.1) | 10 (13) | 0.51 |
| CVA, n (%) | 8 (5.8) | 3 (3.9) | 0.75 |
| Atrial Fibrillation, n (%) | 7 (5) | 5 (6.5) | 0.76 |
| AHI, per h, (IQR) | 6.1 (11.4) | 5.5 (8.9) | 0.76 |
| ODI, per h, (IQR) | 4.1 (7.3) | 3.7 (6.3) | 0.79 |
| T90, %, (IQR) | 15.5 (76) | 14 (82) | 0.89 |
| MeanSpO2, %, (IQR) | 93 (1.7) | 93 (2.4) | 0.74 |
| MinSpO2, %, (IQR) | 86 (6) | 85 (6.5) | 0.37 |
| ESS score, (IQR) | 5.5 (5.3) | 6 (6.0) | 0.9 |
| *Baseline characteristic of participants in relation to ocular parameters*[&] | | | |
| MD, dB, (IQR) | -4.0 (6.6) | -2.7 (6.6) | 0.30 |
| VFI, %, (IQR) | 92 (17) | 93 (19) | 0.78 |
| cRNFL (µm), sd | 66.5 (16) | 72.8 (14.8) | 0.001 |
| CDR, IQR | 0.8 (0.3) | 0.8 (0.3) | 0.71 |

[&] Based on data from both eyes

Definition of abbreviations: HTG=high tension glaucoma, NTG=normal tension glaucoma, BMI=body mass index, IHD=ischaemic heart disease, CVA=cerebrovascular accident, AHI=apnoea-hypopnoea index, ODI=oxygen desaturation index, T90=time spent with oxygen saturation less than 90%, MeanSpO2=mean oxygen saturation, MinSpO2=minimum oxygen saturation, ESS=Epworth Sleepiness Scale, MD=mean deviation, VFI=visual field index, cRNFL=circumpapillary retinal nerve fibre layer thickness, CDR= cup to disc ratio (determined by the optometrist during a slit lamp examination).

Appendix 11. Missing data and exclusions for the analysis of associations between AHI and ocular parameters in the POSAG study cohort.

| Reason for exclusion | POAG group N=235 | Control group N=160 |
|---|---|---|
| Treated OSA, n (%) | 2 (0.85) | 0 (0) |
| Ocular comorbidities, n (%) | 17 (7) | 7 (4.4) |
| Inadequate quality OCT images, n (%) (relevant to all RNFL regression models) | 14 (6) | 7 (4.4) |
| Missing at least one covariate, n (%) (relevant to fully adjusted RNFL regression models) | 13 (5.5) | 15 (9.4) |

Definition of abbreviations: AHI=apnoea hypopnea index, POAG=primary open-angle glaucoma, OCT=ocular coherence tomography, OSA=obstructive sleep apnoea, RNFL= Retinal Nerve Fibre Layer.

Appendix 12. Criterion values and coordinates of ROC curve for the STOP-BANG questionnaire in matched POAG patients ( for AHI≥5, POSAG study).

| Crite rion | Sensitivity | 95% CI | Specificity | 95% CI | +PV | 95% CI | -PV | 95% CI |
|---|---|---|---|---|---|---|---|---|
| >0 | 100 | 96-100 | 1 | 0.03 - 7 | 54 | 53 - 55 | 100 | |
| >1 | 95 | 89 - 99 | 14 | 7 - 24 | 56 | 54- 59 | 71 | 45- 88 |
| >2 | 77 | 66- 85 | 42 | 31 - 54 | 61 | 55 - 66 | 61 | 49 - 71 |
| >3 | 58 | 47 - 69 | 66 | 54- 77 | 67 | 58 - 74 | 58 | 50 - 65 |
| >4 | 27 | 18 - 37 | 84 | 73 - 91 | 66 | 51 - 78 | 50 | 46- 54 |
| >5 | 13 | 7 - 22 | 95 | 87 - 99 | 73 | 48 - 89 | 48 | 46 - 51 |
| >6 | 3 | 1 - 10 | 99 | 93 - 100 | 75 | 24- 97 | 47 | 46- 48 |
| >7 | 1 | 0.03 -6 | 100 | 95 - 100 | 100 | | 47 | 46 - 47 |
| >8 | 0 | 0- 4 | 100 | 95- 100 | | | 46 | 46 - 46 |
| | | | | | | | | |

Appendix 13. Summary of the linear mixed effects models examining the association between OSA status and the rate of global RNFL thinning [$RNFL_{last}$-$RNFL_{baseline}$, µm/year ], (n=167 eyes).

| Model | Estimate | Standard Error | 95% CI | t value | P value |
|---|---|---|---|---|---|
| *Unadjusted:* | | | | | |
| OSA (reference to no OSA) | -0.44 | 0.21 | -0.86 to -0.03 | -2.1 | 0.038 |
| AHI | -0.41 | 0.25 | -0.91 to 0.09 | -1.6 | 0.11 |
| *Adjusted* | | | | | |
| OSA (reference to no OSA) | -0.51 | 0.20 | -0.91to -0.11 | -2.5 | 0.014 |
| CVS co-morbidities (reference to no CVS co-morbidities) | -0.43 | 0.21 | -0.8.5 to -0.002 | -2.0 | 0.049 |
| Baseline RNFL thickness | -0.014 | 0.006 | -0.03 to -0.002 | -2.2 | 0.028 |
| $IOP_{current}$ | -0.064 | 0.028 | -0.12 to -0.009 | -2.3 | 0.024 |
| Previous filtration surgery (reference to no surgery) | -0.30 | 0.25 | -0.79 to 0.19 | -1.2 | 0.23 |
| Corneal Hysteresis | 0.028 | 0.048 | -0.067 to 0.12 | 0.58 | 0.56 |
| Age | 0.007 | 0.012 | -0.017 to 0.03 | 0.57 | 0.57 |

Abbreviations: OSA= obstructive sleep apnoea, Baseline RNFL thickness measured on the first scan included in the analysis, AHI=apnoea-hypopnoea index, $IOP_{current}$=intraocular pressure recorded at the study visit, CVS=cardiovascular co-morbidities (at least one of the following: diabetes, hypertension, ischemic heart disease, atrial fibrillation, cerebro-vascular accident, heart failure, over 20 pack year smoking history).

Appendix 14. Ethical approval for the PAIR 2 study (Chapters 5 and 7)

NHS
Health Research Authority

**East of England - Cambridge South Research Ethics Committee**
The Old Chapel
Royal Standard Place
Nottingham
NG1 6FS

30 March 2016

Dr Ian Smith
Consultant Respiratory and Sleep Physician
Papworth Hospital NHS Trust
Respiratory Support & Sleep Centre
Papworth Hospital
Papworth Everard, Cambridge
CB23 3RE

Dear Dr Smith

| | |
|---|---|
| **Study title:** | **The impact of continuous positive airway pressure on nocturnal intraocular pressure in people with obstructive sleep apnoea with and without primary open-angle glaucoma.** |
| **REC reference:** | **16/EE/0074** |
| **IRAS project ID:** | **201358** |

Thank you for your letter of 29 March 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.

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 Manager, Ellen Swainston, nrescommittee.eastofengland-cambridgesouth@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.*

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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" below).

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The Committee has not yet completed any site-specific assessment (SSA) for the non-NHS research site(s) taking part in this study. The favourable opinion does not therefore apply to any non-NHS site at present. We will write to you again as soon as an SSA application(s) has been reviewed. In the meantime no study procedures should be initiated at non-NHS sites.

**Approved documents**

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

| *Document* | *Version* | *Date* |
|---|---|---|
| Covering letter on headed paper [Response to Research Ethics Committee's Provisional Opinion] | | 20 March 2016 |
| GP/consultant information sheets or letters [Study 2 GP letter ] | 1.0 | 03 February 2016 |
| Instructions for use of medical device [Icare Home manufacturer's manual for patients ] | 1.0 | 09 February 2016 |
| Instructions for use of medical device [CPAP manufacturer's instructions-this is for information only. Not to be provided to the patients] | | 09 February 2016 |
| IRAS Checklist XML [Checklist_29032016] | | 29 March 2016 |
| Letters of invitation to participant [Invitation letter-participants recruited from the POSAG study ] | 1.0 | 03 February 2016 |
| Letters of invitation to participant [Invitation letter-study2-participants recruited from the SDC] | 1.0 | 03 February 2016 |
| Non-validated questionnaire [Patient Post Sleep Questionnaire] | | |
| Other [Emergency contact card for participants ] | 1.0 | 09 February 2016 |
| Participant consent form [Consent form-study 2] | 2.0 | 20 March 2016 |
| Participant information sheet (PIS) [PIS study 2-Participants recruited from the POSAG study ] | 2.0 | 20 March 2016 |
| Participant information sheet (PIS) [PIS study 2-Participants recruited from the SDC] | 2.0 | 20 March 2016 |
| REC Application Form [REC_Form_08022016] | | 08 February 2016 |
| Research protocol or project proposal [Study 2 Protocol ] | 0.2 | 20 March 2016 |
| Summary CV for Chief Investigator (CI) | | 10 November 2015 |

**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

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**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/

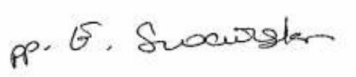
**HRA Training**

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

| **16/EE/0074** | **Please quote this number on all correspondence** |
|---|---|

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

Yours sincerely



**Dr Les Gelling**
**Chair**

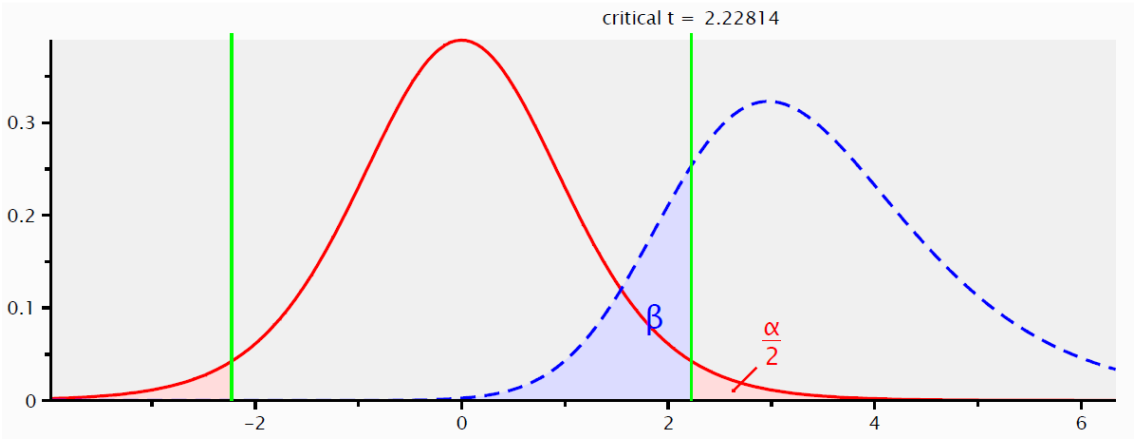
Email: nrescommittee.eastofengland-cambridgesouth@nhs.net

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

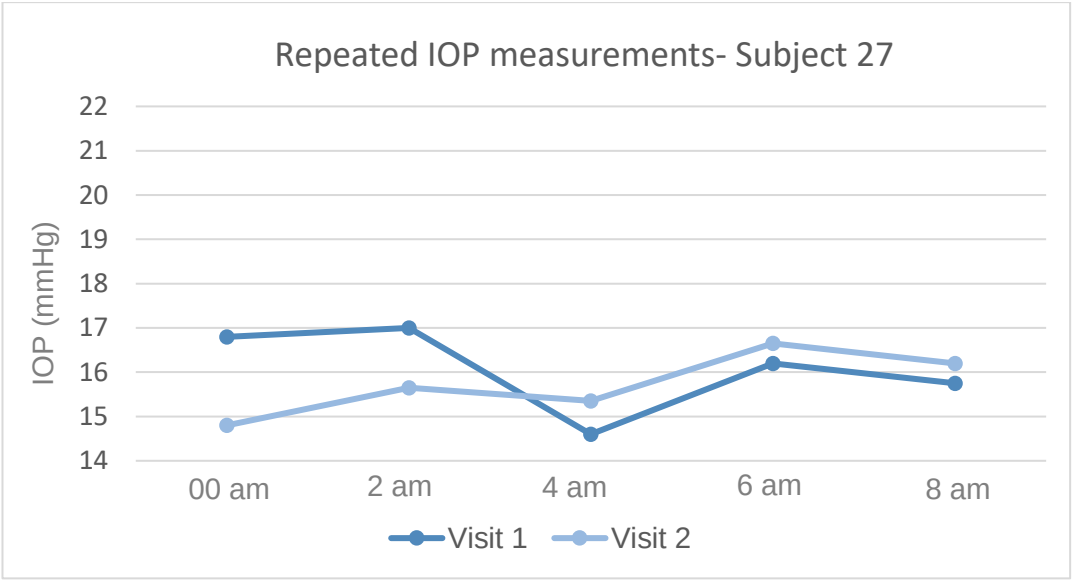
*Copy to:* *Dr Victoria Stoneman*
*Dr Darius Wozniak*

Appendix 15. Sample size calculation for PAIR 2 study (G*Power output).

Saturday, December 12, 2015 -- 13:45:39
t tests - Means: Difference between two dependent means (matched pairs)
Analysis: A priori: Compute required sample size
Input: Tail(s) = Two
Effect size dz = 0.9541603
α err prob = 0.05
Power (1-β err prob) = 0.8
Output: Noncentrality parameter δ = 3.164592
Critical t = 2.228139
Df = 10
Total sample size = 11
Actual power = 0.813361

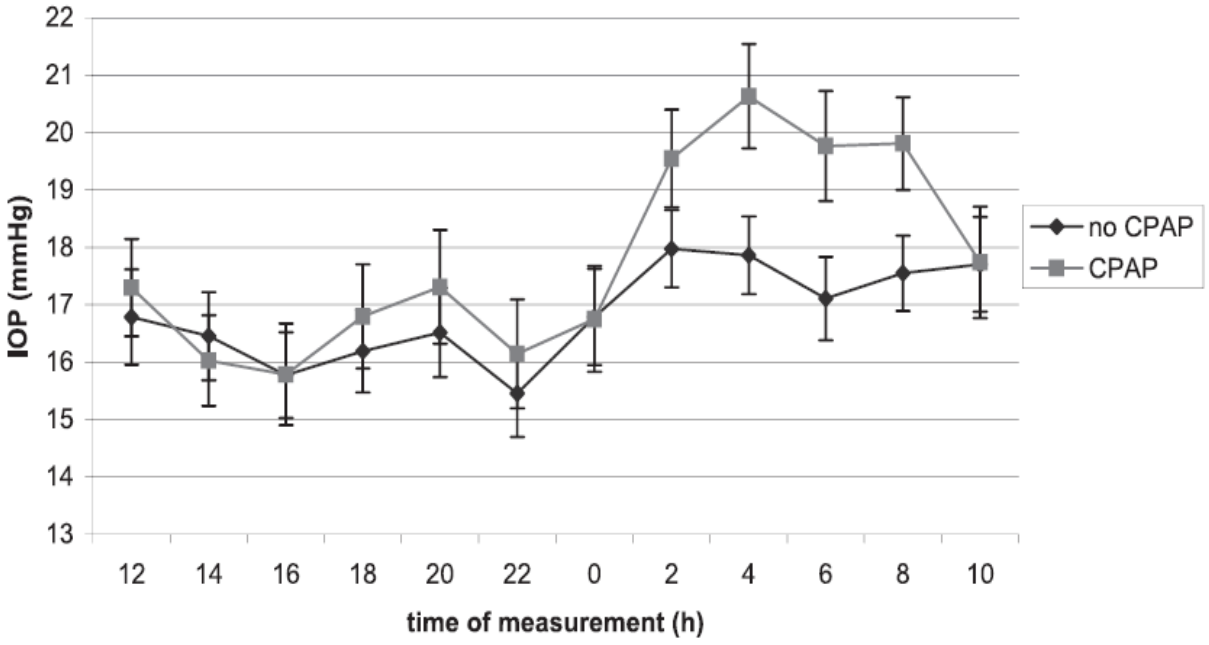



Appendix 16. Repeated IOP measurements from the sleep period at Visit 1 and Visit 2 obtained on a POAG patient (subject 27) who could not tolerate CPAP but attended for both visits and had IOP measured off CPAP on both occasions.



Appendix 17. Average 24-hour IOP in OSA patients at baseline and during CPAP therapy in the study by Kiekens et al.

Adopted from: *Kiekens S, Veva De Groot null, Coeckelbergh T, Tassignon M-J, van de Heyning P, Wilfried De Backer null, et al. Continuous positive airway pressure therapy is associated with an increase in intraocular pressure in obstructive sleep apnea. Invest Ophthalmol Vis Sci. 2008 Mar;49(3):934–40.*



Appendix 18. Three groups of OSA patients with normal, absent and inversed IOP rhythm at baseline and during CPAP therapy according to Pépin et al.

*Adopted from: Pépin J-L, Chiquet C, Tamisier R, Lévy P, Almanjoumi A, Romanet J-P. Frequent loss of nyctohemeral rhythm of intraocular pressure restored by nCPAP treatment in patients with severe apnea. Arch Ophthalmol Chic Ill 1960. 2010 Oct;128(10):1257–63.*

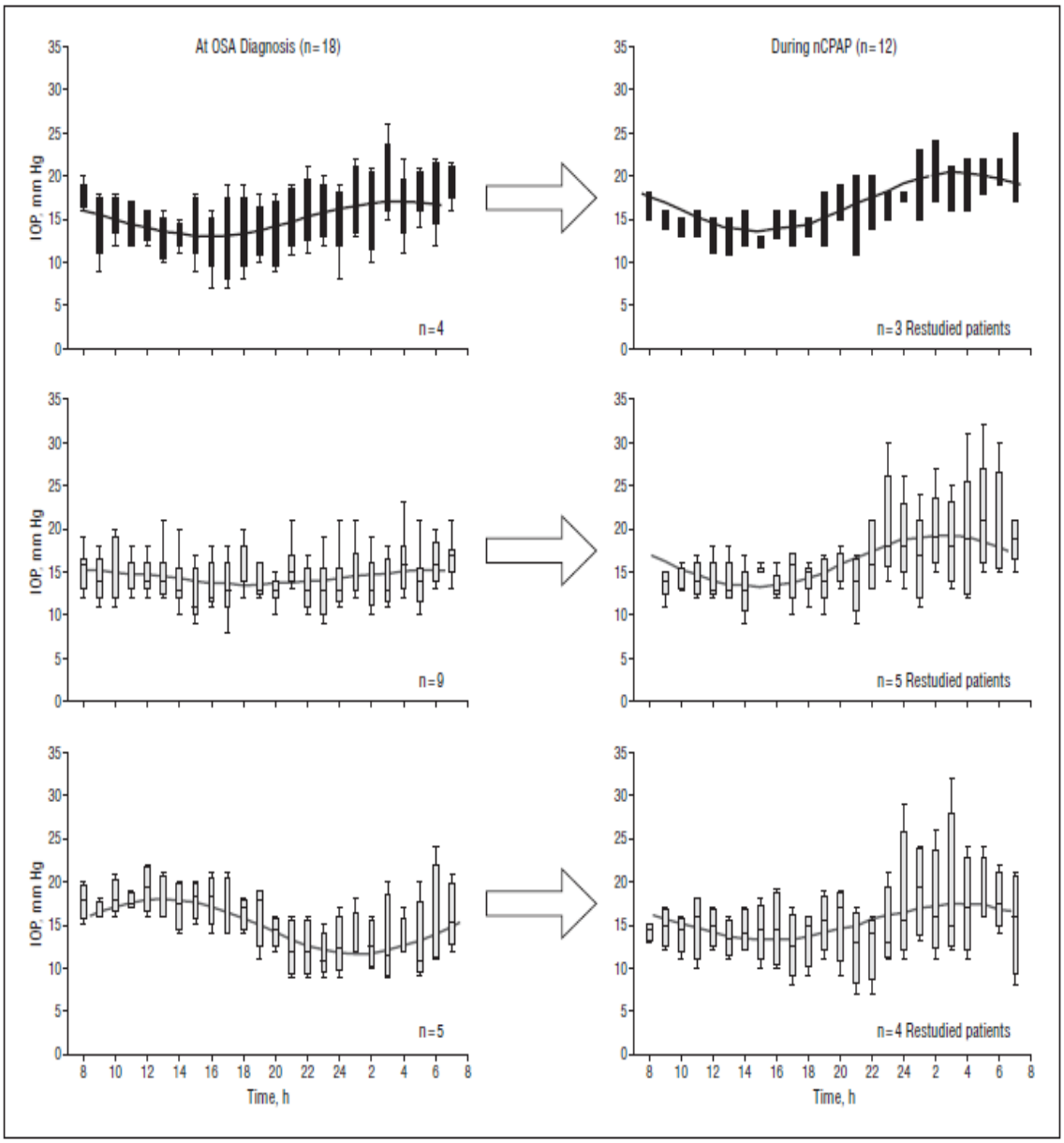



**Figure 1.** Curves of mean (standard error of the mean) intraocular pressures (IOPs) before and during nasal continuous positive airway pressure (nCPAP) treatment of patients with obstructive sleep apnea (OSA). A, At diagnosis, patients had normal IOP nyctohemeral rhythm (nocturnal acrophase); all 3 patients reassessed after treatment continued to have normal IOP rhythm. B, At diagnosis, patients did not have IOP nyctohemeral rhythm; of 5 patients reassessed after treatment, 3 had normal IOP rhythm and 2 no IOP rhythm. C, At diagnosis, patients had IOP nyctohemeral rhythm and diurnal acrophase; of 4 patients reassessed after treatment, 3 had normalized rhythm. The box plot represents the quartiles, extremes, and the median values. The solid line illustrates the fit of the mean IOP data over 24 hours.

Appendix 19. 24-hour IOP rhythm in young healthy subjects and in older people according to a study by Liu et al. Measurements were taken in supine and habitual positions

Adopted from: *Liu JH, Kripke DF, Twa MD, et al. Twenty-four-hour pattern of intraocular pressure in the aging population. Invest Ophthalmol Vis Sci. 1999 Nov;40(12):2912-7.*

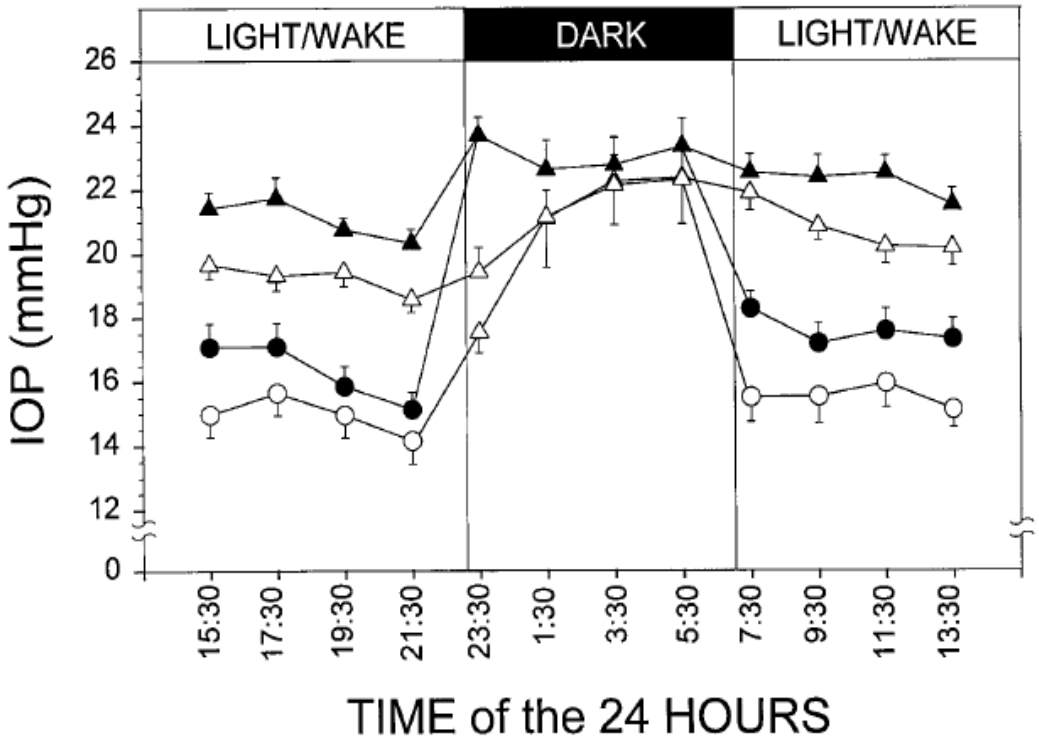



**FIGURE 1.** A comparison of 24-hour patterns of IOPs in the aging and the young volunteers. *Solid symbols* represent a group of 50- to 69-year-old volunteers ($n$ = 21). Intraocular pressure was measured by a pneumatonometer 30 minutes after the odd hours in both the sitting (●) and the supine (▲) positions during the light/wake period (7 AM–11 PM) and only in the supine position during the dark period (11 PM–7 AM). Error bars represent SEM. Previously published results[6] from two separate groups of 18- to 25-year-old volunteers were included for comparison. In one group ($n$ = 12), IOP was measured in the sitting position (○) during the light/wake period and in the other group ($n$ = 21) in the supine position (△). During the dark period, all measurements of IOP were performed with subjects supine. Participants started 24-hour experiments at different times evenly distributed in the light/wake period.

Appendix 20. Ethical approvals for the PAIR 1 study (Chapter 6).

NHS
Health Research Authority

**East of England - Cambridge South Research Ethics Committee**
The Old Chapel
Royal Standard Place
Nottingham
NG1 6FS

04 April 2016

Dr Ian Smith
Consultant Respiratory and Sleep Physician
Papworth Hospital NHS Trust
Respiratory Support & Sleep Centre
Papworth Hospital
Papworth Everard, Cambridge
CB23 3RE

Dear Dr Smith

| | |
|---|---|
| **Study title:** | **Intraocular pressure changes in response to continuous positive airway pressure during diurnal trial in people with treated primary open-angle glaucoma, treatment naÃ¯ve primary open-angle glaucoma patients and control subjects.** |
| **REC reference:** | **16/EE/0073** |
| **IRAS project ID:** | **201356** |

Thank you for your letter of 29 March 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 and Mr Colin Green.

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 Manager, Ellen Swainston, nrescommittee.eastofengland-cambridgesouth@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" below).

Non-NHS sites

The Committee has not yet completed any site-specific assessment (SSA) for the non-NHS research site(s) taking part in this study. The favourable opinion does not therefore apply to any non-NHS site at present. We will write to you again as soon as an SSA application(s) has been reviewed. In the meantime no study procedures should be initiated at non-NHS sites.

**Approved documents**

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| IRAS Checklist XML [Checklist_09022016] | | 09 February 2016 |
| IRAS Checklist XML [Checklist_29032016] | | 29 March 2016 |
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| Letters of invitation to participant [Invitation letter-Study1-Newly diagnosed treatment naive glaucoma patients ] | 1.0 | 03 February 2016 |
| Letters of invitation to participant [Invitation letter-Study 1-participants recruited from the POSAG study ] | 2.0 | 20 March 2016 |
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| Participant consent form [Consent Form ] | | |
| Participant consent form | 2.0 | 20 March 2016 |
| Participant information sheet (PIS) [PIS-study 1-Newly diagnosed glaucoma patients ] | 1.0 | 03 February 2016 |
| Participant information sheet (PIS) [PIS Study 1-Participants recruited from the POSAG study ] | 1.0 | 03 February 2016 |
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| Participant information sheet (PIS) [PIS Study 1-Participants recruited from the POSAG study ] | 2.0 | 20 March 2016 |
| REC Application Form [REC_Form_08022016] | | 08 February 2016 |
| Referee's report or other scientific critique report [Peer-review by Prof. Jonas] | | 21 March 2016 |
| Research protocol or project proposal [Study 1 Protocol ] | 0.1 | 03 February 2016 |
| Research protocol or project proposal | 0.2 | 20 March 2016 |
| Summary CV for Chief Investigator (CI) [IES CV] | | 11 November 2015 |

**Statement of compliance**

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| **16/EE/0073** | **Please quote this number on all correspondence** |
|---|---|

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

Yours sincerely

**Dr Les Gelling**
**Chair**

Email: nrescommittee.eastofengland-cambridgesouth@nhs.net

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

*Copy to:* *Dr Victoria Stoneman*
*Dr Dariusz Wozniak*

Anglia Ruskin University
Cambridge Chelmsford Peterborough

**Ref**: SC/jc/FMSFREP/16/17 011
**Enquiries**: Joanne Corney
**Direct Line**: 01245 684779
**Date**: 15th December 2016

**Chelmsford Campus**
Bishop Hall Lane
Chelmsford
CM1 1SQ
T: 0845 196 4779
Int: +44 (0)1245 493131
www.anglia.ac.uk
**Faculty of Medical Science**

Dariusz Wozniak

Dear Dariusz

**Re: Application for Ethical Approval**

| | |
|---|---|
| **Principal Investigator:** | Dariusz Wozniak |
| **FREP number:** | 16/17 011 |
| **Project Title:** | IOP response to a short-term application of CPAP |

Thank you for your application for ethical approval from the Faculty of Medical Science Faculty Research Ethics Panel (FREP).

Following discussion of your proposal, the Panel decided to offer conditional approval to your project application; the condition being upon the following:

- This study has already been approved by an NHS REC; however, in the participant information sheet the student has been identified as Clinical Research Fellow not a PhD student who is registered at Anglia Ruskin University. The participants and the NHS REC must be informed that this is part of a PhD study at Anglia Ruskin University and the results will be published as part of a thesis.
- Please also clarify the criteria for the termination of the study.

**It will not be necessary for you to resubmit your application but you are required to provide the required document and information stated above.**

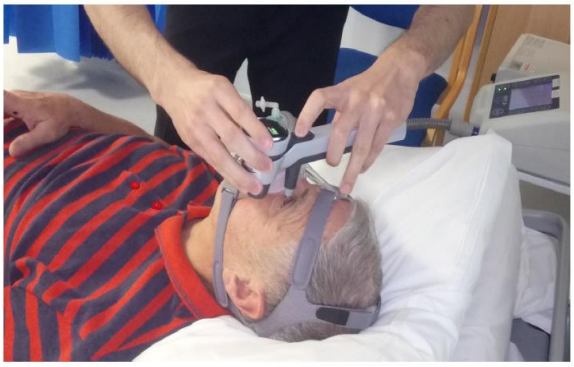
**Please note that under the terms of Anglia Ruskin University's ethics policy you must undertake not to commence your project until you have received full and complete ethics approval.**

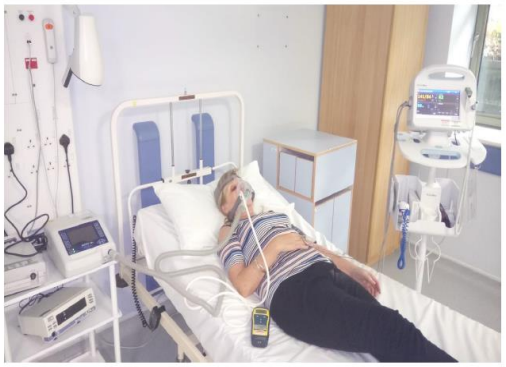
Please do not hesitate to contact me if you have any further queries regarding this matter.

Yours sincerely,

**Prof Selim Cellek**
**Director of Research**
For the Faculty (of Medical Science) Research Ethics Panel

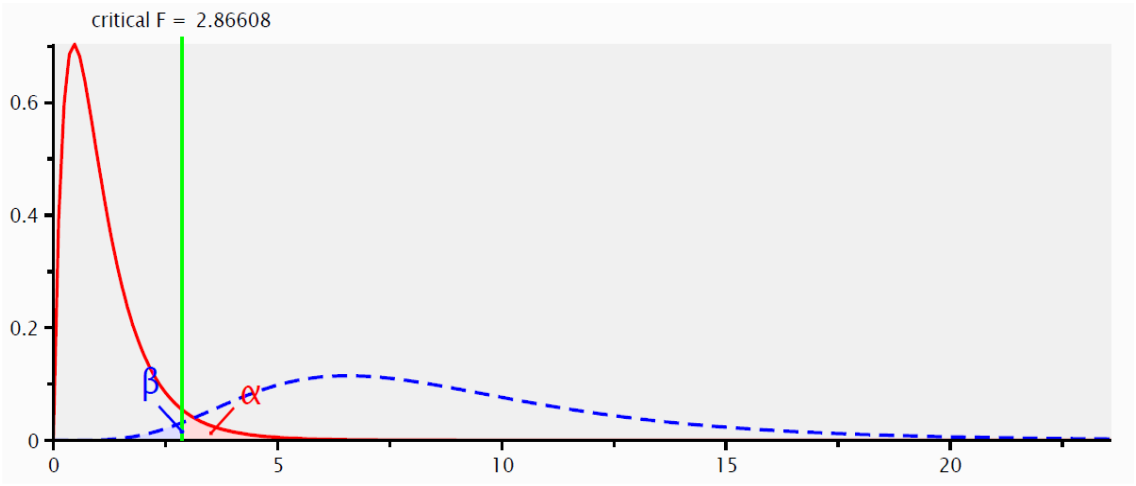
Appendix 21. Illustration of IOP measurement with IcarePro tonometer on two patients who took part in the PAIR I study *(with written consent to share the images).*





Appendix 22. Sample size calculation for PAIR 1 study (G*Power output).

Saturday, December 12, 2015 -- 20:30:40
F tests - ANOVA: Repeated measures, within factors
Analysis: A priori: Compute required sample size
Input: Effect size f = 0.6859943
α err prob = 0.05
Power (1-β err prob) = 0.95
Number of groups = 1
Repetitions = 5
Corr among rep measures = 0.5
Nonsphericity correction ε = 1
Output: Noncentrality parameter λ = 28.235291
Critical F = 2.866081
Numerator df = 4.000000
Denominator df = 20.000000
Total sample size = 6
Actual power = 0.978563




Appendix 23. Examples of different quality ONH and macular OCT-A images provided by the manufacturer.