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VASCULAR AND MORPHOLOGICAL CHANGES OF THE OPTIC NERVE HEAD FOLLOWING THERAPEUTIC INTRAOCULAR PRESSURE REDUCTION IN OPEN ANGLE GLAUCOMA AND OCULAR HYPERTENSION

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Cette thèse intitulée :

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RÉSUMÉ

But de l'étude :

Évaluer les changements vasculaires et morphologiques de la tête du nerf optique (TNO) et de la rétine péripapillaire après thérapie visant à réduire la pression intraoculaire (PIO) chez les patients atteints de glaucome à angle ouvert (GAO) ou d'hypertension oculaire (HTO). La présence et l'étendue des changements au niveau du débit sanguin et de la topographie ont été corrélées avec des paramètres cliniques tel que le rapport excavation/papille, la vasospasticité périphérique et l'épaisseur cornéenne centrale.

Patients et Méthodes:

Vingt patients avec GAO non contrôlés, vingt patients avec HTO non contrôlé et 20 sujets normaux ont été recrutés pour l'évaluation de la fiabilité de la technique et les mesures de référence du débit sanguin.

Pour les patients avec GAO et HTO, la réduction de la PIO a été obtenue par thérapie chirurgicale, laser ou médicamenteuse. Tous les patients ont obtenu une réduction de la PIO de plus de 20% et un suivi minimum de 4 semaines.

La perfusion de la TNO et de la rétine péripapillaire a été évaluée avant et après réduction de la PIO utilisant un logiciel nommé « scanning laser doppler flowmetry (SLDF) full-field perfusion analysis » et permettant d'analyser les images obtenues avec le Heidelberg Retina Flowmeter (HRF). Le débit sanguin moyen a été obtenu à partir de 5 images successives de perfusion. Les mesures topographiques ont été recueillies avant et après réduction de la PIO utilisant le Heidelberg Retina tomograph

(HRT). La topographie moyen a été obtenu a été à partir de 3 images successives et les différences topographiques ont été calculées. De plus, le débit sanguin au niveau d'un doigt a été déterminé grâce au Transonic laser Doppler Flowmeter après immersion dans l'eau froide. L'épaisseur cornéenne centrale a aussi fait l'objet d'une évaluation grâce au pachymètre à ultrasons. La stabilité du champ visuel a été examinée chez une cohorte de patients avec GAO et HTO sur une période de 4 ans en utilisant les critères adaptés de Hodapp, Parrish et Anderson.

Des tests statistiques ont été pratiqués en utilisant l'analyse de variance à 1 facteur et le test T pour données appariées. Les corrélations ont été évaluées grâce au coefficient de Pearson.

Résultats:

Le logiciel d'analyse « full-field perfusion » a peris d'obtenir des données intra- et inter-session hautement reproductibles chez les patients glaucomateux avec une fiabilité ≥0.99 pour l'anneau neurorétinien (ANR) et ≥0.87 pour la rétine péripapillaire.

Les patients avec GAO avant traitement avaient un débit sanguin significativement plus faible que celui des patients avec HTO et des sujets normaux (P=0.001). Chez les patients avec HTO, le débit sanguin de l'ANR etait inversement corrélé au rapport excavation/papille (P=0.039).

Bénéficiant d'un % de réduction similaire de la PIO (37% pour le groupe GAO versus 33% pour le groupe HTO), le débit sanguin de l'ANR s'est amélioré de 67% chez les patients avec GAO (P=0.001) comparé à 7.5% pour ceux avec HTO (p=0.41). Chez ces derniers, l'amélioration du débit sanguin de ANR etait limité aux sujets vasospastiques

(P=0.001). Une corrélation négative et significative (P=0.003) a aussi été trouve entre les changements du débit sanguin dans l'ANR et le débit sanguin maximum au niveau du doigt chez les patients avec GAO contrairement aux patients avec HTO.

Les changements topographiques moyens de la TNO après réduction de la PIO ne différaient pas entre les groupes GAO et HTO étudiés (P≥0.439, ANOVA). Les patients avec les cornées les plus minces présentaient une réduction nettement plus importante de la profondeur moyenne de l'excavation (P=0.003) et de la profondeur maximum de l'excavation (P=0.020) avec, en revanche, de petites améliorations du débit sanguin de l'ANR comparées aux patients ayant les cornées les plus épaisses (P=0.04).

Les patients avec une perte progressive du champ visuel ont été trouvés plus vasospastiques (P=0.006) et montraient une plus grande diminution plus importante de la profondeur maximum de l'excavation (P=0.005). Ils ont aussi des cornées plus minces et une augmentation moins importante du débit sanguin de l'ANR (P≥0.31).

Conclusion:

Cette étude est en accord avec des articles publiés précédemment et mettant en évidence, en l'absence de thérapie oculaire antihypertensive, un débit sanguin diminué chez les patients avec GAO comparés aux patients avec HTO et aux sujets normaux. Les patients avec HTO présentant un plus grand rapport excavation/papille ont aussi montré des débits sanguins de l'ANR plus faibles que ceux présentant des rapports cup/disk plus petits. Cette étude démontre que, pour un pourcentage similaire de réduction de la PIO, le débit sanguin de l'ANR chez les sujets glaucomateux s'améliore alors qu'il reste stable chez les patients présentant une hypertonie oculaire. Considérant

ces derniers, de plus grandes améliorations du débit sanguin de l'ANR ont été observées chez les sujets vasospastiques que chez les sujets non vasospastiques.

Aucun changement topographique de la TNO ne montre une différence statistique significative entre les deux groupes étudiés avant et après thérapie oculaire antihypertensive. Cependant, les patients avec GAO et HTO présentant les cornées les plus minces ont montré un déplacement plus important de la lame criblée comparés à ceux ayant les cornées les plus épaisses.

L'évaluation de la stabilité visuelle à long terme d'une cohorte pilote de nos patients avant la baisse de PIO initiale montre que les patients glaucomateux présentant une progression étaient plus vasospastiques et présentaient une lame crible plus compliante comparés aux sujets avec un déficit stable.

Ces résultats démontrent l'existence d'un débit sanguin de la TNO altéré dans le glaucome et établissent des liens entre la neuropathie optique glaucomateuse et des facteurs tels que l'autorégulation vasculaire, la vasospasticité périphérique, l'épaisseur cornéenne centrale et la compliance biomécanique de la tête du nerf optique.

Mots Clés

Glaucome - tête du nerf optique - débit sanguin - pression intraoculaire - topographie - vasospasticité - épaisseur cornéenne centrale - autorégulation.

ABSTRACT

Purpose:

To evaluate the vascular and morphologic changes of the optic nerve head (ONH) and peripapillary retina following therapeutic intraocular pressure (IOP) reduction in open angle glaucoma (OAG) and ocular hypertension (OHT).

The presence and extent of blood flow changes and topographic changes were correlated with clinical parameters such as cup/disc ratio as well as peripheral vasospasm and central corneal thickness. A correlation between the ONH changes and long term visual field stability was also studied.

Patients and Methods:

Twenty uncontrolled OAG patients, 20 uncontrolled OHT patients and 20 normal volunteers were recruited for assessment of the reliability of the technique and for baseline blood flow measurements.

For both OAG and OHT groups, IOP reduction was achieved by medical, laser or surgical therapy. All patients had IOP reduction more than 20% and a minimum of 4 weeks followup.

ONH and peripapillary retinal perfusion was assessed before and after IOP reduction using scanning laser Doppler flowmetry (SLDF) full-field perfusion image analysis of Heidelberg Retina Flowmetry (HRF) images. Mean flow values were derived from five consecutive perfusion images. Scanning laser topographic measurements were performed before and after IOP reduction using the Heidelberg Retina Tomograph (HRT). Mean topography of 3 consecutive images was obtained and topography

differences were computed. Patients also underwent finger blood flow measurement using the Transonic laser Doppler flowmetry after cold water immersion, while central corneal thickness was determined using an ultrasound pachymeter. Visual field stability was monitored in a cohort of the OAG and OHT patients over a period of 4 years using modified Hodapp-Anderson-Parrish criteria.

Statistical evaluations were performed using one-way analysis of variance and two-tailed distribution paired T-test. Correlations were assessed using Pearson's coefficient.

Results:

SLDF full-field perfusion analysis was found to be highly reproducible in glaucoma patients both within and between sessions with a reliability of ≥ 0.99 in the neuroretinal rim and ≥ 0.87 in the peripapillary retina.

For baseline flow, OAG patients had significantly lower blood flow in the ONH compared with OHT patients and normal volunteers (P=0.001). Among patients with OHT, neuroretinal rim blood flow was inversely correlated to increased C/D ratio (P=0.039).

Following similar % IOP reduction (37% in OAG versus 33% in OHT), ONH neuroretinal rim blood flow improved by 67% in OAG (P=0.001) compared to 7.5% in OHT (P=0.41). In OHTs, improvement in neuroretinal rim blood flow was limited to vasospastic subjects (P=0.01). A significant negative correlation (P=0.003) was also found between rim blood flow change and maximum finger blood flow in OAG patients but not in OHTs.

Mean change in ONH topographical parameters following therapeutic IOP reduction did not differ between the two study groups (P≥0.439, ANOVA). Patients with thinner corneas had greater reductions in mean cup depth (P=0.003) and maximum cup depth (P=0.020) and smaller improvements in neuroretinal rim blood flow compared to those with thicker corneas (P=0.04).

Patients with progressive visual field changes were found to be vasospastic (P=0.006) and showed shallowing of maximum cup depth (P=0.005). They also had thinner corneas and smaller increases in neuroretinal rim blood flow (P≥0.31).

Conclusion:

The study confirms previous reports as regards decreased baseline ONH blood flow in OAG patients compared to OHT patients and normal subjects. Ocular hypertensives with larger cup/disk ratios were also shown to have lower neuroretinal rim blood flow compared to those with smaller C/D ratios. The study demonstrates that following a similar percentage of therapeutic IOP reduction, blood flow improved in the neuroretinal rim of the ONH in glaucoma patients while it remained stable in ocular hypertensives. Greater improvements in rim blood flow were observed in vasospastic than in non-vasospastic ocular hypertensives.

None of the ONH topographic changes between the two study groups showed a statistically significant difference following therapeutic IOP reduction. However, OAG and OHT patients with thinner corneas showed greater forward displacement of the lamina cribrosa compared to those with thicker corneas.

Assessment of long-term visual stability in a pilot cohort of our subjects showed that at the time of initial IOP reduction, progressive glaucoma patients were more vasospastic and demonstrated a more compliant lamina cribrosa compared to stable glaucoma patients.

The results demonstrate the role of defective ONH blood flow in glaucoma and correlate glaucomatous optic neuropathy to factors such as autoregulation, central corneal thickness and peripheral vasospasm. The results also shed light on the mechanical properties of the ONH following sustained IOP reductions in a real-life context. Comparing these vascular and morphologic changes in both OAG and OHT helps the assessment of long-term visual stability.

Key Words

glaucoma - optic nerve head - blood flow - intraocular pressure - topography - vasospasticity - central corneal thickness - autoregulation.

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LIST OF ABREVIATIONS

ACE	Angiotensin Converting Enzyme
AFFPIA	Automatic Full-Field Perfusion Image Analysis
ANOVA	Analysis of Variance
AU	Arbitrary Units
CCT	Central Corneal Thickness
C/D ratio	Cup to Disc ratio
CDI	Color Doppler Imaging
CLBF	Cannon Laser Blood Flowmeter
CRA	Central Retinal Artery
CSLO	Confocal Scanning Laser Ophthalmoscopy
CSM	Cup Shape Measure
DBP	Diastolic Blood Pressure
DSFS	Doppler Shift Frequency Spectrum
EDV	End Diastolic Velocity
FA	Fluorescein Angiography
FPA	Fundus Pulsation Amplitude
GON	Glaucomatous Optic neuropathy
HRF	Heidelberg Retinal Flowmeter
HRT	Heidelberg Retinal Tomograph
HTG	High Tension Glaucoma
HVC	Height Variation Contour
ICG	Indocyanine Green

IOP	Intraocular Pressure
LDF	Laser Doppler Flowmetry
LDV	Laser Doppler Velocimetry
MD	Mean Defect
NTG	Normal Tension Glaucoma
OA	Ophthalmic Artery
OAG	Open Angle Glaucoma
OBF	Ocular Blood Flow
OCT	Optical Coherence Tomography
OHT	Ocular Hypertension
ONH	Optic Nerve Head
OPP	Ocular Perfusion Pressure
PCA	Posterior Ciliary Artery
PCV	Peak Systolic Velocity
POBF	Pulsatile Ocular Blood Flow
RBC	Red Blood Cell
RGC	Retinal Ganglion Cell
RI	Resistivity Index
RNFL	Retinal Nerve Fiber Layer
RNFLA	Retinal Nerve Fiber Layer Area
RNFLT	Retinal Nerve Fiber Layer Thickness
ROI	Region of Interest
RPE	Retinal Pigment Epithelium

RVA	Retinal Vessel Analyzer
SBP	Systolic Blood Pressure
SD	Standard Deviation
SLDF	Scanning Laser Doppler Flowmetry
SPCAs	Short Posterior Ciliary Arteries
TPU	Tissue Perfusion Units
VF	Visual field

Dedication

To my father and to the memory of my mother who taught me the first step to success…persistence...
To my wife Nora for her endless help, support, patience and motivation…
And to the most precious gift in my life.....my two sons Omar and Ziad….

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Chapter 1

Introduction and Review of Literature

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ANATOMY AND BLOOD SUPPLY OF THE OPTIC NERVE HEAD

The optic nerve head (ONH), also known clinically as the optic disc or papilla, forms the point of exit for the retinal ganglion cell (RGC) axons through the scleral canal. It is composed primarily of neural fibers (1.2-1.5 million retinal ganglion cell axons), glial cells, extracellular matrix supportive tissue and vascular elements (1-7). The ONH is delineated from the adjacent peripapillary tissue by a scleral rim of connective tissue, the border tissue of Elschnig (8). The diameter of the ONH and anterior portion of the optic nerve is approximately 1.5 mm (9).

The ONH may be divided conveniently into four anatomic regions, from front to back (10-16):

A. Surface nerve fiber layer: this region is continuous with the nerve fiber layer of the retina. It is composed of the non-myelinated axons of the RGCs in transition from the superficial retina to the neuronal component of the optic nerve.

B. Prelaminar region: this is the region between the surface nerve fiber layer and the lamina cribrosa, at the level of the choroid and outer retina. It consists of nerve fibers arranged in bundles, surrounded by glial tissue septa and astrocytes.

C. Lamina cribrosa region: lies adjacent to the sclera, and provides the main support for the optic nerve as it exits the eye and penetrates the scleral coat.

D. Retrolaminar region: this region lies immediately posterior to the lamina cribrosa. It is marked by the beginning of axonal myelination and is surrounded by the leptomeninges of the central nervous system.

Differences among these four regions reflect the conditions to which the axons are exposed to as they pass through the ONH. These differences include axon myelination posterior to the lamina cribrosa, sources of blood supply, and the change in tissue pressure from intraocular pressure to that of the cerebrospinal fluid.

Anatomy of the Lamina Cribrosa

The lamina cribrosa is a complex collagenous, relatively elastic structure that consists of a series of fenestrated sheets of connective tissue (approximately 10) and provides the main support for the axons of the optic nerve (1,10-12). The sheets of the lamina cribrosa span the scleral opening at the back of the eye inserting into the outer half of the sclera. They are arranged in a series of parallel stacked plates.

Each of these sheets contains fenestrations or pores that are vertically aligned to allow the passage of the neural elements of the optic nerve. Central pores allow transit of the central retinal artery and central retinal vein. In humans, the pores of the lamina cribrosa are histologically larger and fewer superiorly and inferiorly and the laminar sheets are thinner when compared to the nasal and temporal aspects of the optic nerve (17) (Figure 1.1). This correlates with the reported preferential loss of axons at the superior and inferior poles of the ONH in glaucomatous optic neuropathy (GON). Enlargement of the laminar pores and alteration of the shape of cup both peripherally and posteriorly are

characteristic signs of glaucomatous optic neuropathy (11). Glaucomatous changes are thus not only characterized by neural tissue loss, but also by connective tissue changes resulting in distortion and posterior bowing of the lamina cribrosa.

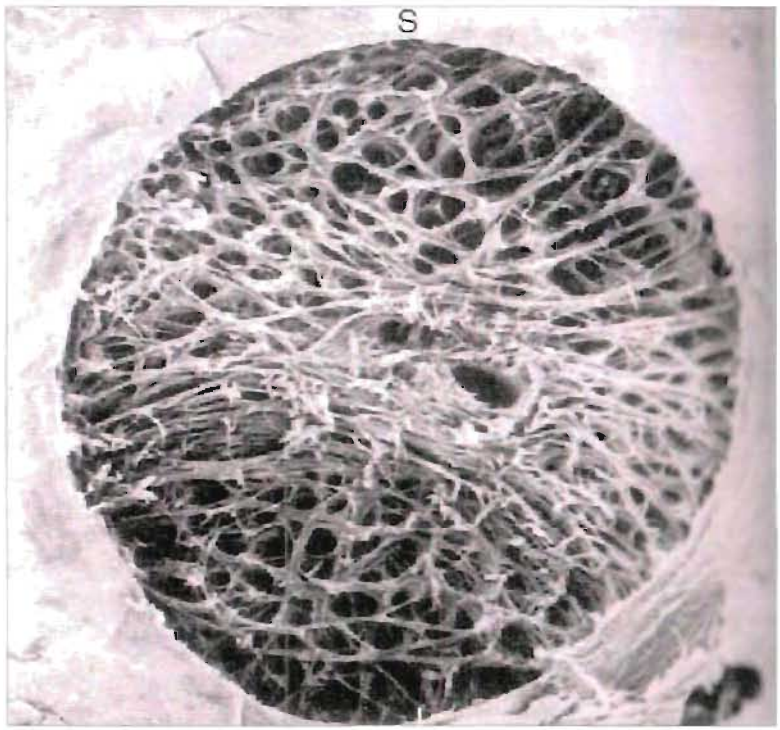

Figure 1.1: Scanning electron microscopic analysis of a normal human ONH following trypsin digestion. Thinner and less dense laminar sheets are noted in the superior and inferior region of the ONH. Courtesy Dr H. Quigley

Lamina cribrosa sheets are composed primarily of collagen as well as extracellular matrix components including elastin, laminin and fibronectin. In adults, laminar sheets possess large amounts of type-I collagen required for rigidity and resistance to deformation as well as type-3 collagen and elastin required for elasticity and compliance (18,19). Such composition with both supportive and elastic elements is

thought to have an impact on the behavior of the lamina in response to IOP elevation or fluctuation. Laminar sheets are also lined with astrocytes which provide an interface between the sheets and the nerve fiber bundles as well as provide metabolic support for the axons (20).

General Anatomy of the ONH Blood Supply

The arterial supply of the ONH is derived entirely from branches of the ophthalmic artery, a branch of the internal carotid artery (13-16).

Typically between 2-4 posterior ciliary arteries arise from the ophthalmic artery in the posterior orbit and course anteriorly before dividing into approximately 10-20 short posterior ciliary arteries. Often the posterior ciliary arteries separate into a medial and lateral group before branching into short posterior ciliary arteries. The short posterior ciliary arteries penetrate the sclera surrounding the optic nerve. They anastomose to form a circle approximately 100-300 microns posterior to the suprachoroidal space, called the arterial circle of Zinn-Haller (21). This circle supplies the peripapillary choroid, as well as most of the anterior optic nerve.

The central retinal artery (CRA), also a branch of the ophthalmic artery, penetrates the optic nerve 10-15 mm behind the globe but has few if any intraneural branches apart from a small branch within the retrolaminar region (Figure 1.2).

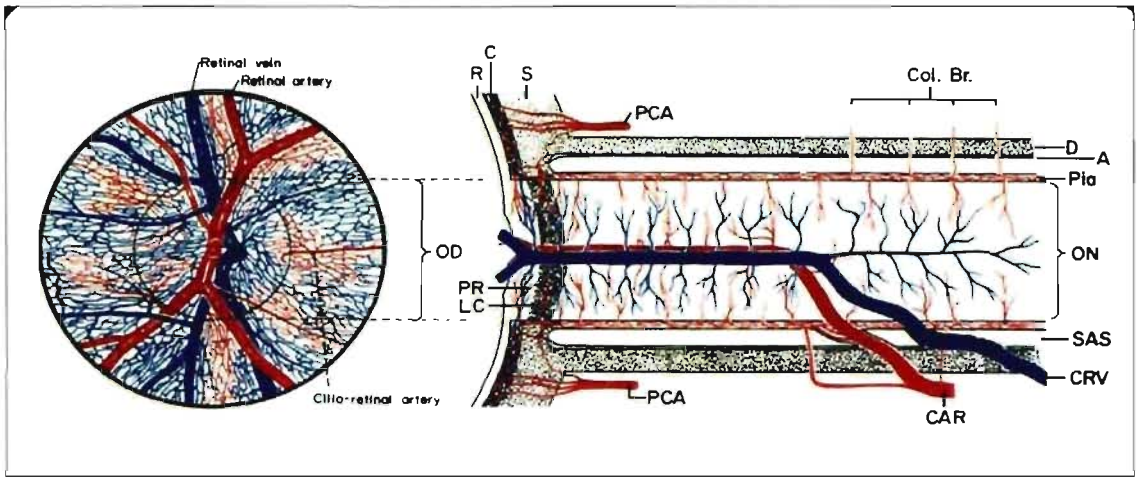

Figure 1.2: Schematic representation of blood supply of the optic nerve. Reprinted from Hayreh SS: Trans Am Acad Ophthalmol Otolaryngol 1974; 78:240-254. CAR: central retinal artery, CRV: central retinal vien, PCA: posterior ciliary artery, LC: lamina cribrosa, PR: prelaminar region, ON: optic nerve, R: retina, C: choroid S: sclera, OD: optic disc, D: Dura, A: Arachnoid.

Microvasculature of specific ONH regions

The blood supply of the optic nerve head can best be discussed under its four regions, from front to back (13-16) (Figure 1.3):

A. Surface nerve fiber layer: This layer is mostly supplied by recurrent retinal arterioles from the CRA and its branches. As the CRA emerges from the optic nerve, it branches into a superior and inferior trunk. From these major trunks as well as from the more distal branches, small arterioles supply the surface nerve fiber layer of the optic nerve and peripapillary retina. The cilioretinal artery, when present, usually supplies the corresponding sector of the surface layer.

B. Prelaminar region: This region is supplied by branches from the short PCAs (posterior ciliary arteries) either directly or through the arterial circle of Zinn-

Haller. Some investigators have reported that branches from the short PCAs may course through the choroid and supply the prelaminar region (22). The CRA provides no branches in this area.

C. Lamina cribrosa region: This region is similarly supplied by branches from the short PCAs either directly or through the arterial circle of Zinn-Haller. The CRA gives no branches in the lamina. Blood vessels in the lamina cribrosa are 10-20 microns in diameter, are situated in the fibrous septa and form a dense capillary plexus which makes this part of the ONH highly vascular.

D. Retrolaminar region: This region is also supplied by branches from the short PCAs as well as by the pial branches originating from the CRA before it pierces the retrobulbar optic nerve.

In summary, except for the branches of the surface nerve fiber layer that arise from the CRA, the occasional pial branches of the CRA and the minor contribution from the choroidal vasculature to the prelaminar and laminar regions, the principal arterial supply to the ONH is derived from the short posterior ciliary arteries.

Corrosion casts and histological studies on enucleated human eyes (23) (Figure 1.3) demonstrated the capillary bed of the ONH as one continuous network, communicating anteriorly with the capillary network of the retina and posteriorly with that of the rest of the optic nerve. The CRA contribution to the surface nerve fiber layer becomes intertwined with the short PCA contribution to the more posterior regions of the ONH.

Such anastomosis of capillaries could be viewed as a protective mechanism against ischemia.

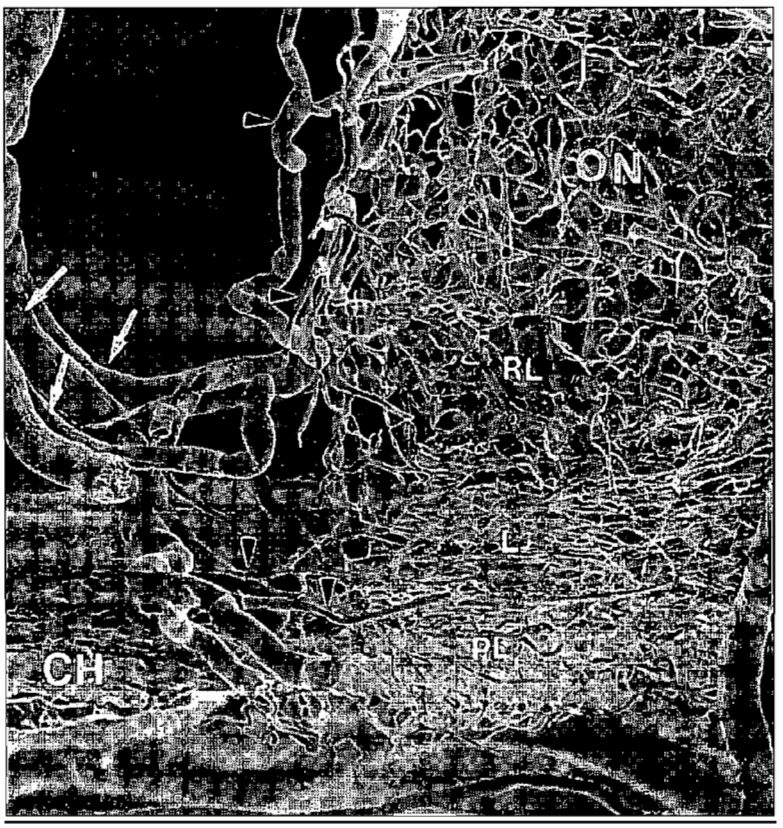

Figure 1.3: Microvascular corrosion cast of human optic nerve showing posterior ciliary arteries (arrows) and pial arteries (arrowheads). ON: optic nerve, L: laminar region, RL: retrolaminar region, PL: prelaminar region, CH choroid. Reprinted from Onda EO, Cioffi GA et al. Am J Ophthalmol 1995; 120: 92

Variations in Blood Supply of the ONH

The vascular anatomy of the ONH has been extensively studied. Yet the precise microvasculature of this region remains difficult to ascertain because of the small vessel caliber, the complex three-dimensional architecture and the relative inaccessibility of

the tissues. Inter-individual variation in anatomic details also has led to confusion and controversy.

The existence of the arterial circle of Zinn-Haller, the contribution of the peripapillary choroid to the ONH circulation and the longitudinal anastomosis of capillaries throughout the regions of the ONH are all examples of the persisting debates.

Inter-individual variations in the anatomical distribution and blood flow patterns of PCAs were described by Hayreh and coworkers (14) and could be summarized as:

1. Variations in the number of PCAs and their subdivisions: Prior to entering the sclera, the main PCAs (ranging from 1 to 5 PCAs arising from the ophthalmic artery) subdivide into multiple branches which consist of two subgroups: short and long PCAs. The short PCAs in turn consist of two subgroups: paraoptic and distal. Evidence suggests that the ONH is mostly supplied by the paraoptic short PCAs (24).

2. Variations in the area of supply of each PCA: In vivo experimental and clinical fluorescein angiographic studies have shown marked variations in the area of supply of each PCA. Studies have also revealed a segmental distribution with no anastomoses between the adjacent segments (25) and a marked inter-individual as well as interocular variation in the areas supplied by each PCA (26).

3. Variations in location of watershed zones in the PCA vascular bed: In vivo studies have shown watershed zones in the border between the territories of distribution of PCAs (27). In the event of a drop in ocular perfusion pressure, the watershed zones become most vulnerable to ischemia. The location of the PCA watershed zone in relation to the ONH may be an important factor in determining the site and severity of glaucomatous optic neuropathy.

Venous Drainage of the ONH

The venous drainage from the ONH is by the central retinal vein and its tributaries (4). In the prelaminar region the ONH also has connections with the peripapillary choroid. In the laminar and retrolaminar regions, some venous drainage may also be via pial veins and ultimately into the central retinal vein as it exits the optic nerve.

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BACKGROUND AND DEFINITION OF GLAUCOMA

Glaucoma is the leading cause of irreversible blindness worldwide (1), affecting an estimated 67 million individuals of whom approximately 10 percent are bilaterally blind. It is the number two cause of blindness and visual handicap in Canada affecting 1% of the Canadian population (2). The prevalence of glaucoma rises dramatically with age increasing exponentially particularly after the age of 40 (3). While the prevalence of glaucoma in Caucasians between 40 and 50 years is around 0.9%, it increases to 2.1% in patients over 80 years old (4) and to 8.8% in the elderly population of African descent (5).

Primary open angle glaucoma is by far the most common form of the disease in North America. Primary open angle glaucoma is defined as a chronic generally bilateral and often asymmetrical disease which is characterized in at least one eye by all of the following (6-9):

1. Evidence of glaucomatous optic nerve damage from either or both of the following:
    a. appearance of the disc or retinal nerve fiber layer (e.g. thinning or notching of the disc rim, progressive change, nerve fiber layer defects)
    b. characteristic abnormalities in the visual field (e.g. arcuate defect, nasal step, paracentral scotoma, generalized depression) in the absence of other causes
2. Adult onset
3. Open normal-appearing anterior chamber angles

4. Absence of known other causes of open angle glaucoma

Elevated IOP remains a major risk factor for OAG, with an exponential increase in disease prevalence and incidence associated with increasing levels of IOP (9). Recent evidence from several multi-centered studies support the role of pressure lowering in glaucoma therapy (10-16).

Elevated IOP alone, however, cannot explain the pathogenesis of glaucoma. Numerous studies have demonstrated that as many as 50% of newly diagnosed cases of glaucoma have IOP within normal limits, and that a good percentage of these will never develop elevated IOP (normotensive glaucoma). Furthermore, up to 95% of individuals identified with IOP above normal levels do not have glaucoma, and will not develop the disease over time, even when left untreated (ocular hypertension). IOP reduction however remains the only proven method of reducing the number of individuals who will eventually develop glaucoma after four years from 9.5% to 4.4% (17).

Since the dominant risk factor for the development and progression of glaucoma is IOP, management of glaucoma involves reduction of IOP to a target level by medical, laser or surgical intervention. The target IOP is established by reducing the maximal untreated IOP by either 30% or to 21 mmHg whichever is lower. Additional IOP reduction could be indicated for eyes with advanced cupping and visual field loss. Treated patients are thus observed for progression of cupping and field changes which if occurs is an indication for additional or alternative intervention to lower IOP (18).

Conversely, several other risk factors other than IOP have been identified in glaucoma (19-24). Some of these risk factors have been related to decreased ocular perfusion specifically chronic ischemia, atherosclerosis, vasospasm or defective autoregulation. Others have been related to abnormalities in corneal, scleral and ONH structure, specifically altered lamina cribrosa compliance in response to fluctuation of IOP.

Until recently,. there had been a lack of noninvasive techniques to routinely and precisely assess the integrity of the vascular perfusion of the ONH and peripapillary retina, areas where most of the damage associated with glaucoma typically occurs. Recent technical advances have enabled noninvasive and reliable quantification of ultra-fine details of ONH topography (via confocal scanning laser ophthalmoscopy) and blood flow (via scanning laser Doppler flowmetry).

Using such techniques, a large body of epidemiologic, clinical and experimental evidence suggest that defective ocular blood flow is associated with glaucoma and involved in its pathogenesis (25-32). Substantial in vivo and ex vivo clinical studies also suggest that pressure-sensitive changes to the mechanical properties of the ONH and specifically to the lamina cribrosa have been invoked as contributing to glaucomatous optic neuropathy (33-38).

To date, the precise nature of such ONH biomechanical changes and the related defects in ocular blood flow remain unclear.

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ROLE OF INTRAOCULAR PRESSURE AND OCULAR BLOOD FLOW IN THE PATHOGENESIS OF GLAUCOMA

Although the clinical picture of glaucoma is well described, the exact mechanism leading to this specific type of damage to the optic nerve head (ONH) is not yet clear. As previously mentioned it is generally accepted that the mechanism of damage in glaucoma is almost certainly multifactorial (1).

While elevated IOP remains the risk factor most commonly associated with glaucomatous optic neuropathy (GON), numerous other variables involved in the development and progression of glaucoma have been identified (2-7). Vascular risk factors in particular have been extensively studied (8,9). These include systemic blood pressure alterations (10-12), diabetes (13,14), reduced ocular blood flow (OBF) (15-18) and vasospasm (19-24).

Conventionally, two theories have been presented for the pathogenesis of glaucoma, pressure (25) and vascular (26):

A- Pressure theory, introduced by Muller, supposes that GON is a direct consequence of elevated IOP, damaging the lamina cribrosa and neural axons.

B- Vascular theory, suggested by von Jaeger, considers GON as a consequence of insufficient blood supply to the ONH due to either elevated IOP or to other risk factors reducing OBF.

Both theories are not mutually exclusive. In fact, they are believed to act in synergism, but discussing them separately helps to demonstrate their respective roles.

A- Role of Intraocular Pressure in Glaucoma

The pressure theory hypothesizes that elevated IOP leads to elongation, stretching and collapse of the ONH tissues and in particular the lamina cribrosa. Plates of the lamina cribrosa demonstrate posterior rotation with misalignment of the laminar pores.

Axons of the retinal ganglion cells passing through the laminar pores can be damaged either directly by compression, kinking or tissue deformation or indirectly through disruption of axoplasmic transport (27,28). Retrograde axoplasmic transport is essential for the delivery of many substances necessary for the survival of the retinal ganglion cell bodies. Interruption of this process could trigger pathways that lead to retinal ganglion cell (RGC) death via apoptosis (28). Obstruction of axoplasmic transport at the level of the lamina cribrosa in response to elevated IOP has been demonstrated in the primate glaucoma model using radioactive tracers (29,30).

This pressure theory is consistent with studies showing that elevated IOP causes posterior bowing of the lamina cribrosa which can disrupt its organization. The hypothesis also correlates the characteristic pattern of optic nerve damage in glaucoma with the anatomy of the lamina cribrosa. The regions with the greatest damage in the superior and inferior poles of the ONH correspond to those areas of the lamina cribrosa that have the thinnest laminar beams or the least connective tissue density.

Both experimental as well as clinical studies have proven the role of IOP and the benefits of IOP lowering therapy in glaucoma. Yet therapeutic IOP reduction was

shown to improve the prognosis of patients but does not always stop progression of the disease. The existence of NTG on one hand and OHT on the other indicates that other factors might be involved in the pathogenesis of GON by rendering the eye more sensitive to IOP changes.

B- Role of Ocular Blood Flow in Glaucoma

The vascular theory proposes that glaucomatous optic neuropathy is due to insufficient perfusion of the ONH predominantly at the level of the lamina cribrosa resulting in ischemic damage. Defective autoregulation and abnormal vasospastic responses could account for differences in susceptibility to IOP-induced damage.

Findings of Ocular Blood Flow Studies in Glaucoma and their Interpretation

Investigations using epidemiologic, histological and non-invasive clinical techniques point to defective ocular blood flow as an important risk factor in glaucomatous optic neuropathy (15-18). Such hypoperfusion of the ONH was reported to be caused by atherosclerosis, vasospasm and vascular changes related to movement of the lamina cribrosa.

In general, studies have reported slower ocular blood flow velocities in glaucoma patients compared to normals. Blood flow velocities have been found to be lower in the

retina, ONH and choroid as well as in the retro-ocular vessels and in the peripheral circulation. The fact that the reduction of ocular blood flow has often been observed to precede the damage and that blood flow can also be reduced in other parts of the body of glaucoma patients, suggests that the hemodynamic alterations may at least partially be primary (31). Also, given the probable variability in the vascular mechanisms, the observed variability in reduction of blood flow is expected.

Studies have also shown that glaucoma patients are more likely to demonstrate ocular as well as systemic vascular changes compared to normal subjects. Such observations could well point to an underlying vascular mechanism for glaucomatous optic neuropathy (GON):

1- Ocular Vascular Changes in Glaucoma:

A number of ocular signs point indirectly to the fact that at least in some glaucoma patients, blood flow plays an important role. Changes in conjunctival capillaries (e.g. perilimbal aneurysms), localized constriction of peripapillary retinal arteries (32), increased prevalence of disc hemorrhages (33) preservation of nerve fibres around retinal vessels (34) and the possible significance of cilioretinal arteries (35) have all been described in glaucoma patients (Figure 1.4).

Studies have also shown that glaucoma patients are twice as likely to have crescent-shaped RPE and/or choroidal atrophic changes at the disc margin which might be attributed to ischemia (36).

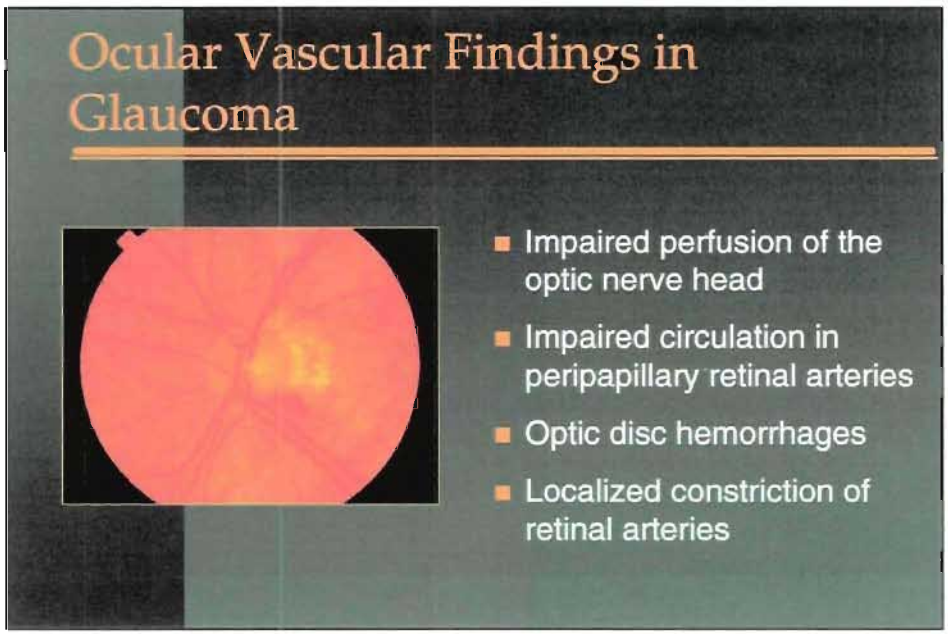

Figure 1.4: Ocular vascular findings in glaucoma including disc hemorrhages, localized constriction of peripapillary arteries and peripapillary atrophy.

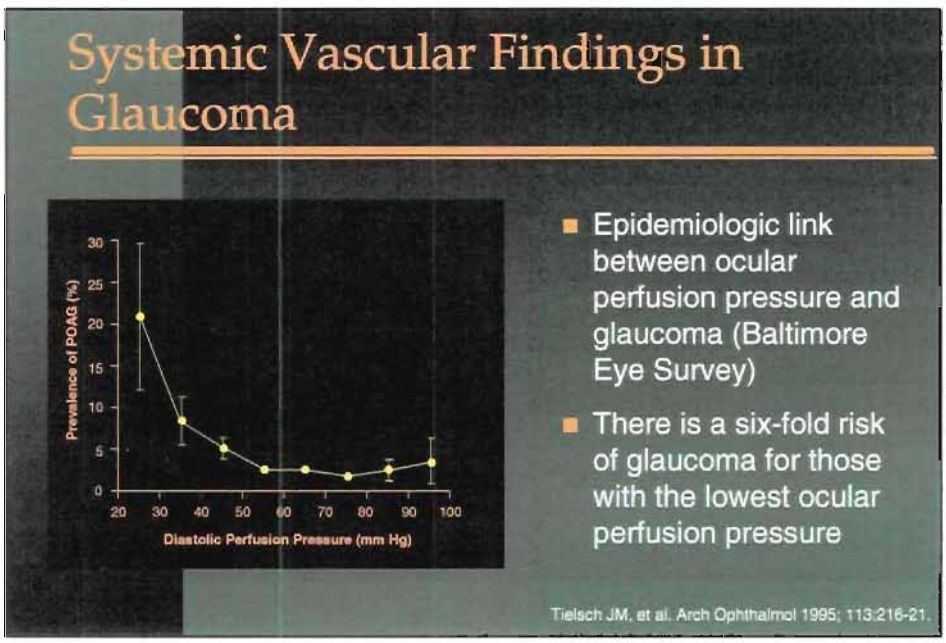

Figure 1.5: Systemic vascular findings in glaucoma showing a six-fold increase in the prevalence of glaucoma in those with lowest ocular perfusion pressure. Courtesy Dr Tielsch et al.

2- Systemic Vascular Findings in Glaucoma:

Epidemiologic links have been reported between ocular perfusion pressure (OPP) and glaucoma [Baltimore Eye Survey (10), Rotterdam Eye Study (37), Egna-Neumarkt Eye Study (38), Barbados Eye Study (39)]. In the Baltimore Eye Survey, there was a six-fold risk of glaucoma for those with the lowest ocular perfusion pressure (Figure 1.5).

The Collaborative Normal Tension Glaucoma Study (40) demonstrated a highly significant association between the rate of progression and the presence of migraines in normal tension glaucoma patients.

Other studies have also reported exaggerated nocturnal blood pressure dips in OAG and NTG patients with progressive field loss. It was hypothesized that such dips might compromise perfusion of the ONH (12,41).

Findings of ocular blood flow studies in glaucoma are difficult to interpret for various reasons (31): authors use different techniques and therefore measure different aspects of ocular circulation; they include glaucoma patients at different stages e.g. early versus late; different types of glaucoma are studied e.g. normal tension glaucoma (NTG) versus high tension glaucoma (HTG); some studies include provocation tests while others do not. Consequently, the interpretation of the available data is difficult as blood flow reduction may, at least partly, be secondary to a reduced demand.

Furthermore, blood flow alterations have been described in various parts of the ocular circulation and it remains unclear how circulatory disorders in parts of the eye other than the anterior optic nerve may affect survival of axons and retinal ganglion cells.

Finally, the influence of additional factors such as systemic blood pressure, vasospasm, vascular dysregulation and plasma levels of vasoactive agents such as endothelin remain to be clarified.

Potential Mechanisms of Ocular Blood Flow Reduction in Glaucoma Patients

Theoretically, there are three components to ocular blood flow reduction in glaucoma patients (31):

A- Increased local resistance to flow, B- Decreased ocular perfusion pressure (OPP), C- Increased blood viscosity.

Several indications point to the role of both increased local resistance to flow and decreased ocular perfusion pressure in glaucoma:

A- Local resistance to flow: increased resistance to flow is manifested as reduced vascular diameter and is affected by either structural changes as anatomic variations in the vessels, vasculitis, or mechanical obstruction of the lumen (via thrombosis or arteriosclerosis) or functional changes such as defective autoregulation of blood flow. Reduced vascular diameter can also be due to reversible spasm of the smooth muscle cells in the vessel wall.

B- Ocular perfusion pressure: OPP equals mean arterial blood pressure minus venous pressure in a specific vascular bed. Normally venous pressure is slightly higher than

IOP, and for practical purposes IOP is a good indicator of the venous pressure. Therefore, OPP can be considered as the difference between mean arterial blood pressure and IOP (where mean arterial blood pressure = Diastolic blood pressure + 1/3 [systolic blood pressure – diastolic blood pressure]).

During the past four decades an increasing amount of evidence has supported the theory that defective perfusion of the ONH plays a crucial role in the pathogenesis of glaucomatous optic neuropathy.

Studies by Hayreh (42-44) have shown a close association between glaucomatous optic neuropathy and systemic vascular disorders as hypertension, hypercholesterolemia, cardiovascular diseases and diabetes. Hayreh (45) hypothesized that seretonin released from carotid, ophthalmic and posterior cerebral arteries in atherosclerotic patients produces transient vasospasms of the ONH and thus contributes to the development and progression of glaucomatous optic neuropathy (GON) and in particular NTG. In their analysis of optic nerve blood flow abnormalities in glaucoma, Flammer and Orgul (31) considered arteriosclerosis as a less important factor for the increased local resistance to blood flow that contributes to defective optic nerve perfusion. Although experimental studies by Hayreh et al (46) indicated that arteriosclerosis might increase the sensitivity to IOP elevations and although some arteriosclerotic patients were shown to present with a sclerotic type of GON (45), Flammer and Orgul (31) believed there was currently very little evidence linking GON to arteriosclerosis or its risk factors (gender, obesity,

hypercholesterolemia, smoking, diabetes, hypertension, carotid stenosis). They attributed increased local resistance to ocular blood flow to a functional rather than a structural change, namely to an abnormal or defective autoregulation of ONH blood flow. Autoregulation refers to the capacity of an organ or tissue to regulate its blood supply in accordance to its functional or metabolic needs. With intact autoregulation, changes in ocular perfusion pressure or metabolic demands are associated with local constriction or dilatation of the terminal arterioles which causes vascular resistance to increase or decrease, thereby maintaining a constant supply of oxygen and nutrients. Conversely, abnormal autoregulation could be expressed not only as an excessive arterial constriction (vasospasm) but also as an inadequate arterial dilatation (26,47). Observations by Drance and coworkers (24) have confirmed an increased prevalence of vasospasm in patients with NTG.

Optic nerve blood flow was also reported to be influenced by ocular perfusion pressure. Reduced ocular perfusion pressure might be due to increased IOP or decreased systemic blood pressure.

NTG patients were reported to have a clearly increased prevalence of systemic hypotension (41). Lower systemic blood pressure, both systolic (48) and diastolic (11), was also found, particularly during the night, in patients with progressive glaucoma compared to stable patients (Figure 1.6). This association between glaucomatous damage and low blood pressure has been confirmed by several authors (49,50).

Consequently, there is little doubt that low systemic blood pressure is an essential risk factor as is increased IOP.

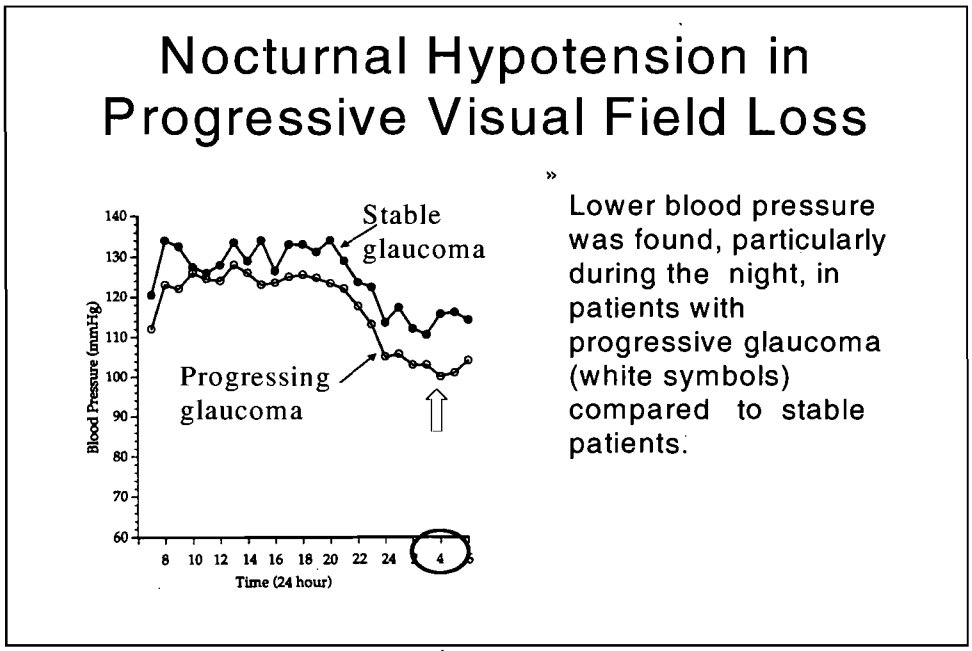

Figure 1.6: Nocturnal hypotension in progressive visual field loss. Lower levels of blood pressure were found, particularly during the night, in patients with progressive glaucoma (arrow) compared to stable glaucoma patients. Courtesy Dr. Meyer et al.

Increased systemic blood pressure on the other hand was reported to shift the autoregulatory plateau to a higher level compared to normals. This adaptation improves the person's tolerance to hypertension but at the same time makes the individual less tolerant to low systemic blood pressure and more susceptible to an immediate and permanent damage from ischemia. Consequently, patients with chronic systemic hypertension are considered to be at greater risk for cerebral or coronary ischemia as well as for GON when subjected to reduced ocular perfusion pressure (11).

In general, epidemiologic studies suggest that systemic hypertension is protective against glaucoma in younger patients (presumably through improved OPP) but deleterious in older patients (presumably through atherosclerosis or loss of autoregulation) (10).

Finally, the contribution of hypercoagulability states to GON has been investigated by several authors. Drance et al (1) found a relative hyperviscosity in NTG patients although this was not confirmed by subsequent publications (51,52). A recent study by O'Brien et al (51) reported activation of coagulation cascades and fibrinolysis pathways in untreated OAG compared with controls. Hamard et al (52) using a laser Doppler velocimeter found decreased blood flow and increased red cell aggregability in NTG. It can be concluded that although as yet there is no consistent evidence as to the presence of an abnormal rheology in NTG, the presence of abnormalities should be considered within each NTG patient.

Current Evidence of Abnormal Ocular Blood Flow in Glaucoma:

Evidence that defective perfusion of the ONH plays an important role in the pathogenesis of GON has been accumulating over the past four decades. However, it is only recently that technical advances have made possible the quantification of blood flow in the different intraocular tissues as well as in both retrobulbar and peripheral circulations. The following section reviews briefly the current evidence of the role of

defective perfusion of the ONH, retina and choroid as well as the various factors involved in the pathogenesis of GON.

A. Impaired optic nerve head, retinal and choroidal blood flow in glaucoma

Some of the main evidence implicating blood flow deficits in glaucoma is derived from fluorescein angiography (53-55). These studies have shown delayed retinal circulation as well as impaired perfusion of the ONH, peripapillary retina and choroid in glaucoma patients. The severity of perfusion defects progresses with the severity of glaucoma and the defects correlate well with visual field loss and nerve fibre layer dropouts (54,55). Techniques using color Doppler imaging (56-58) and pulsatile ocular blood flow (59-61) have demonstrated that both retrobulbar blood flow and bulk choroidal blood flow are reduced in glaucoma patients in contrast to normal subjects.

Using single-point laser Doppler flowmetry, several authors reported decreased blood flow in the ONH of OAG when compared to control subjects (62) and to glaucoma suspects (63).

Scanning laser Doppler flowmetry (SLDF) was also used for several comparisons between flow measurements in glaucoma patients and normal subjects. Michelson et al (64) reported that both neuroretinal rim blood flow and peripapillary retinal blood flow were significantly decreased in OAG patients. Neuroretinal rim blood flow was less by 71% whereas peripapillary retinal flow was less by 49%. Findl and associates (18) reported reduced flow in both the disc cup (-46%) and the neuroretinal rim (-18%) in

OAG patients whereas Nicolela et al (65) reported a significant decrease in flow in the lamina cribrosa but not in the neuroretinal rim of OAG patients compared to normals. SLDF has also been used to compare ONH and retinal perfusion between glaucoma patients and ocular hypertensives. Kerr and associates (66) reported reduced blood flow in the lamina cribrosa and the temporal neuroretinal rim of the ONH of OAG patients in comparison to OHT patients.

B. Improvements in ocular blood flow following therapeutic IOP reduction

Ocular perfusion has been evaluated in glaucoma patients and ocular hypertensives following therapeutic IOP reduction.

The ability of topical antiglaucoma medications to alter ocular perfusion has been reported by several authors, using different methods to assess ONH and retinal blood flow as well as retrobulbar circulation (67-71).

Investigators have also reported improved ocular perfusion following sustained IOP reduction in glaucoma patients following surgery. Color Doppler imaging demonstrated significant improvements in retrobulbar hemodynamics following trabeculectomy (72) whereas pulsatile ocular blood flow measurements similarly showed a significant increase (29%) following reduction of IOP post-surgery (73).

C. Blood flow responses to an induced change in IOP using suction cup

The ability of the eye to adjust to a sudden increase in IOP was thoroughly investigated both in experimental animals as well as in humans. Blood flow responses to an induced

change in IOP using a suction cup have been studied in animal models and in man using laser Doppler flowmetry (74), color Doppler imaging (75), and SLDF (76).

In general, suction-induced IOP elevations reduced retrobulbar, retinal and ONH perfusion parameters in normal and glaucomatous eyes. Such hemodynamic changes were reversed following normalization of the IOP.

Induced changes using suction cup have also been used to demonstrate the highly-dependent relationship between the hemodynamics of the central retinal artery and the short posterior ciliary arteries with acute changes in IOP (75). Acute incremental elevation of IOP in healthy humans resulted in a progressive drop in both central retinal artery and short posterior ciliary arteries flow velocities implying a close link between mechanical and hemodynamic factors in the ONH. In contrast, ophthalmic artery flow velocities were found to be unaffected by such changes.

D. Correlation between reduced neuroretinal rim blood flow values and large C/D ratio

A significant inverse correlation was reported by Michelson and associates (64) between decreased neuroretinal rim blood flow and C/D ratio in glaucoma patients. A similar correlation was reported by Piltz-Seymour et al (63) in their evaluation of optic nerve head perfusion in glaucoma suspects. Higher flow values were reported in OAG suspects with smaller C/D ratio compared to those with larger C/D ratio.

These correlations suggest a link between defective perfusion of the ONH and the severity of glaucomatous optic neuropathy.

E. Defective Autoregulation of the Optic Nerve Head Blood Flow in Glaucoma

As previously mentioned, autoregulation maintains a relatively constant blood flow in spite of changes in ocular perfusion pressure (OPP) which in turn depends on systemic blood pressure and IOP. In the absence of an intact autoregulation, there is an inverse relationship between IOP and OPP. The higher the IOP the lower the OPP and consequently, the lower is the blood flow to the ONH. On the other hand, reduction of IOP would be expected to improve ocular perfusion pressure and consequently increase ONH blood flow.

Changes in OPP occur routinely in daily life as mediated by stress or exercise-induced elevations in systemic blood pressure, by nocturnal reductions in systemic blood pressure and by diurnal variations in IOP (10). When such changes in OPP occur, local constriction or dilatation in the terminal retinal arterioles causes vascular resistance to increase or decrease, thereby maintaining constant blood flow and nutrient supply to the tissues (42).

The existence of intact autoregulation in the normal ONH has been demonstrated in a large number of experimental (77-79) as well as clinical (80-83) studies. Autoregulation has been reported to operate only within a critical range of ocular perfusion pressure and becomes ineffective when the ocular perfusion pressure goes below or above this critical range. This range of ocular perfusion pressure has been investigated in different species and using various methodologies. In healthy monkeys, Geijer and Bill (77)

reported ONH autoregulation to be normal at an ocular perfusion pressure of >30 mmHg. Ernest (83) reported similar findings with pressures >50 mmHg. Breakdown of autoregulation was reported to take place at an ocular perfusion pressure of <25 mmHg by Sossi and Andersen (78), at <30 mmHg by Sperber and Bill (79) and at 30-35 mmHg ocular perfusion pressure by Hayreh and coworkers (46).

Substantial evidence in the literature suggests that GON maybe due to an eventual breakdown in autoregulation.

Ernest (83) speculated that GON maybe due to breakdown in the autoregulatory mechanism that normally keeps the blood flow at levels adequate for tissue requirements. He further speculated that such an autoregulatory mechanism may be damaged by systemic diseases. Sossi and Anderson (78) speculated that such defective autoregulation maybe acquired with age. Pillunat et al (84) presented evidence of abnormal autoregulation in the ONH whereas Grunwald et al (85) reported evidence of abnormal autoregulation in macular blood flow of glaucoma patients.

In a recent study Riva et al (86) reported an increase in ONH blood flow, as measured by laser Doppler flowmetry, of 39.0% in normals versus only 17.5% in ocular hypertensives and 10.4% in early glaucoma patients when the fundus was stimulated with a 15Hz monochromatic green light flicker. Flicker stimulation was reported to increase metabolic demands and consequently induce an increase in blood flow via vasodilatation mediated by nitric oxide release.

It has been generally assumed that the choroid does not possess the capacity to autoregulate. However, recent studies provided evidence that the choroid has some autoregulatory capacity in response to changes in ocular perfusion pressure in healthy subjects. In OAG patients, such autoregulation was considerably impaired while in OHT patients the autoregulation was found to be normal, increased or slightly decreased (87).

F. Effect of Inhaled Carbon Dioxide on Retrobulbar Circulation in Glaucoma

Earlier studies on the response of retinal circulation to changes in arterial oxygen and carbon dioxide were limited to measurements of vessel diameter. These studies reported that hyperoxia decreased retinal vessel diameter whereas hypoxemia increased it (88).

Later Riva et al (89), using laser Doppler velocimetry, measured changes in retinal blood velocity in normal subjects following induced hyperoxia. After 5 minutes of oxygen breathing, blood velocity was reduced by 53%, vessel diameter by 12% and calculated flux by 60%. Similar results were reported by other investigators (90,91).

Studies using blue-field entoptic stimulation also found decreased velocities of perimacular leukocytes associated with hyperoxia and increased velocities with hypoxemia (92). Increased velocities were reported by Sponsel et al (93) using the same technique and with mixtures of 95% oxygen and 5% carbon dioxide.

Induced gas perturbations were also used to test the hypothesis that glaucoma patients show pre-existing and reversible vasoconstriction of retrobulbar vasculature and thus differ from normals in their response to vasoactive stimuli. In a study by Harris et al (56), CDI was performed on the eyes of NTG patients and control subjects, before and after breathing carbon dioxide (CO2). Baseline values for end-diastolic velocities were found to be lower in the ophthalmic arteries of NTG patients compared to those of healthy subjects and the resistivity index was found to be higher. When PCO2 was increased, controls remained unchanged whereas end-diastolic velocity increased in NTG. Similar findings were reported by Hosking et al (94).

These studies suggest the presence of a relative vasoconstriction in some orbital vessels of glaucoma patients. The vasoconstriction might be the result of vasospasm and is partially reversed by hypercapnia.

G. Role of Vasospasm in the Development and Progression of Glaucoma

A high prevalence of peripheral vasospasticity has been reported in glaucoma patients. This vasospasticity has been consistently linked to abnormal ocular blood flow.

Phelps and Corbett in 1985 (95) were the first to suggest the possible role of vasospastic phenomena in the development and progression of GON. They found that 47% of their patients with NTG also suffered from migraine. Gasser and Flammer (19) described ocular vasospasm in which patients with unexplained scotomas had abnormal

capillaroscopic response to cold in the nailfold of the fingers. The scotomas were aggravated by the immersion of the hand in cold water. The authors assumed that patients with a tendency to vasospasm exhibit ocular vascular reactions similar to those that occur in the capillaries of the fingers. In 1988, Guthauser et al (22) demonstrated statistically significant relationship between patient's history of cold hands and the outcome of both the visual field cold water test and the nailfold capillaroscopic test. The visual field results were also found to correlate significantly with the capillaroscopic results.

Strong associations have also been established between NTG and migrainous headaches. Drance et al (24), using Doppler blood flow measurements in the finger and a cold test, showed that in non-glaucomatous subjects, 26% without migraine had a positive vasospastic response while 64% with classic migraine showed such a response. Of the patients with low-tension glaucoma, 65% showed a positive vasospastic response. These findings were later supported by results from the Collaborative Normal Tension Glaucoma Study (40) that demonstrated a 2.58-fold increased risk of progression in glaucoma patients suffering from migraines.

Studies also suggested a possible role for calcium channel blockers in patients with progressive NTG and an underlying vasospastic disorder. Kitazawa et al (96) demonstrated improvement in visual fields following treatment by nifedipine for 6 months whereas Netland et al (97) looked retrospectively at NTG patients on calcium

channel blockers and found that they were less likely to progress. Pillunat et al (98) reported that NTG patients showed increased ocular pulse amplitude and improved central visual fields during rebreathing of carbon dioxide, a known vasodilator.

The relationship between vasospastic changes and structural ischemic changes in glaucoma remains not fully understood, though it has been repeatedly suggested in the literature that insufficient vasospastic regulations in the ONH could explain the abnormal ONH autoregulation. Schulzer and coworkers (99) presented evidence of two distinct populations of OAG patients: patients that were predominantly vasospastic and those that were predominantly atherosclerotic. Vasospastic OAG patients showed high correlation between the amount of visual field damage and the highest IOP while in those with atherosclerosis, no such correlation was found (Figure 1.7).

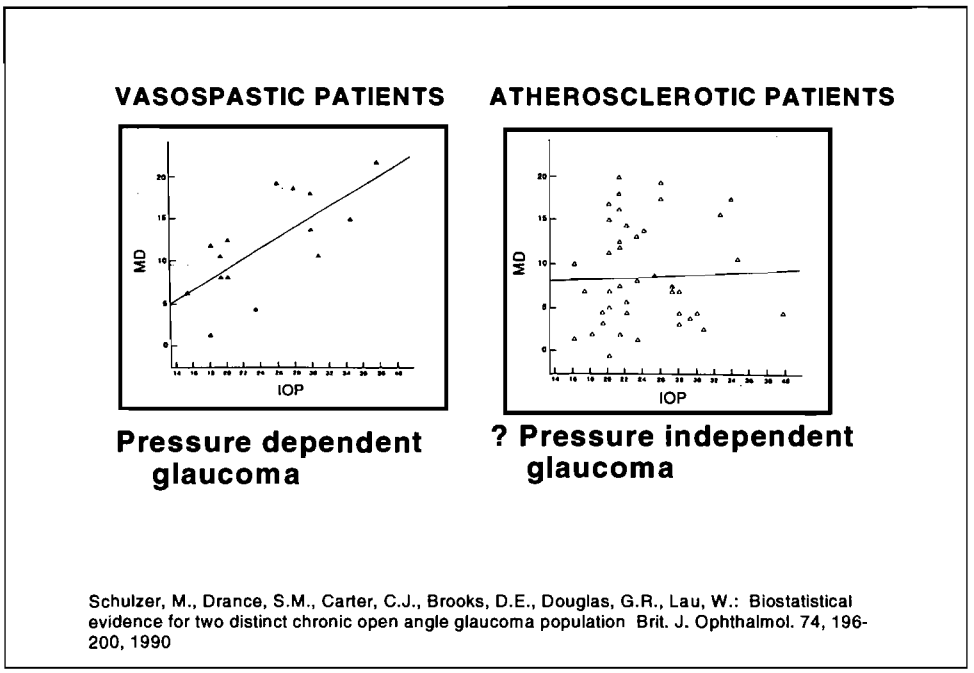

Figure 1.7: Correlations between IOP and visual field mean defect MD in two distinct OAG populations; vasospastic and atherosclerotic. Courtesy Dr. Schulzer et al.

Conclusion

In summary, clinical, epidemiological and experimental data consistently links abnormal ocular perfusion to OAG. The vast majority of published studies on ocular blood flow find reduced ocular perfusion in glaucoma patients. Blood flow decreases with increase in damage, and this reduction occurs both in early and later stages of glaucoma. The reduction in blood flow involves different parts of the eye, including the ONH, choroid and retinal circulation, as well as retrobulbar and even peripheral blood flow. Blood flow alterations are more pronounced in normal-pressure glaucoma than in high-pressure glaucoma and in progressive than in non-progressive eyes. In studies applying provocation tests, differences between OAG patients and normal subjects were more pronounced under provocation.

Current evidence also suggests that abnormalities in ocular blood flow can be explained partly by low ocular perfusion pressure, and partly by vasospasm and abnormal autoregulation of blood flow, which can manifest as an inability to adapt to increased or fluctuating IOP or to decreased systemic blood pressure or to both.

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TECHNIQUES USED FOR EVALUATION OF OCULAR BLOOD FLOW

Vascular risk factors in the eye may have an important role in the pathogenesis of glaucomatous optic neuropathy. A primary requirement for understanding such risk factors is the ability to precisely and reliably evaluate the state of ocular perfusion in health and disease. This has led to the exploration of many innovative and diverse techniques. The fact that the different methods available measure different aspects of ocular circulation and at different locations in the eye complicates direct comparisons between techniques. In this chapter we review the principle, validity, advantages and limitations of the various methods used to measure ocular blood flow.

A- COLOR DOPPLER IMAGING

Color Doppler imaging (CDI) combines ultrasound imaging with Doppler shift analysis to measure blood flow in the retrobulbar vasculature. The technique evaluates blood flow velocity by detecting shifts in the frequency of sound reflected from the flowing blood.

CDI focuses primarily on velocities in the ophthalmic artery (OA), central retinal artery (CRA) and short posterior ciliary arteries (SPCAs), specifically those feeding the nasal and temporal sides of the ONH. From knowledge of retrobulbar vascular anatomy as well as an understanding of the characteristic waveform of the Doppler signal, specific

retrobulbar vessels can be located, and from the Doppler spectrum of these vessels the direction of blood flow is identified (1). Blood flowing away from the center of the body towards the CDI probe is generally arterial and is displayed in red whereas blood flowing towards the center of the body and away from the probe is venous and is displayed in blue. Flow velocity data are plotted against time. The peak and trough of the waves are then identified. From these points peak systolic velocity (PSV) and end diastolic velocity (EDV) are measured (Figure 1.8). These parameters can be used to calculate the resistivity index. The resistivity index (RI) is the most reproducible parameter of the CDI and is calculated as RI = (PSV - EDV) / PSV.

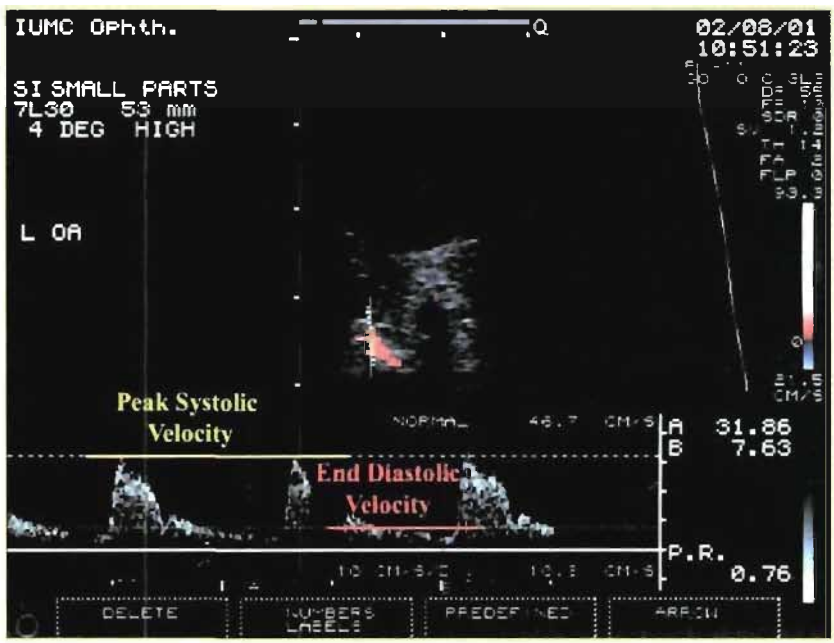

Figure 1.8: Color Doppler imaging showing peak systolic and end-diastolic velocities in the ophthalmic artery (courtesy Dr Alon Harris)

CDI studies have found reduced peak systolic velocity and end-diastolic velocity and increased resistivity index in the retrobulbar vessels of OAG patients (2) and NTG patients (3) when compared with normal controls. Lower baseline retrobulbar blood flow velocities were found in eyes with progressive visual field damage and uncontrolled intraocular pressure (4). Conversely, reduction of IOP after trabeculectomy was shown to result in a significant improvement in retrobulbar blood flow parameters (5).

Advantages:

The technique is attractive and relatively easy to understand. Results can be obtained reliably from the larger vessels such as the ophthalmic or central retinal arteries. The widespread presence of color Doppler machines further encourages pursuing it as a useful investigative tool for the retrobulbar vasculature.

Limitations:

a- CDI allows measurement of velocity rather than blood flow. With no information about vessel diameter, it is not possible to determine the blood flow in a particular vessel. Localized narrowing of vessels was also reported to produce an increase in velocity despite decrease in blood flow (6).

b- The reliability of CDI results depends on the caliber of the vessel imaged and the angulation of the Doppler probe relative to it. A high level of location-dependent variability has been reported for measurements from the CRA (7). Reproducible probe

positioning is subject to the skill and experience of the user and comes after a relatively long learning curve.

c- It is not clear whether the calculated resistivity index (RI) correlates with true vascular resistance *in vivo*.

d- The resolution of CDI might not allow precise measurement of the blood velocity within the small vessels supplying the ONH (e.g. short posterior ciliary arteries, SPCAs) (8). The variability of measurements in these arteries was reported to be higher compared to the OA or the CRA.

e- In the retrobulbar region, short posterior ciliary arteries are varied in their number, size and position from eye to eye (9) and lie intertwined with one another, which makes it difficult to detect by CDI which of those short posterior ciliary arteries is being measured and which actually supplies the ONH.

f- Abnormal velocities and resistivity in the ophthalmic artery does not mean reduced blood flow in the ONH, because blood flow in the ophthalmic artery does not necessarily correlate with that in the short posterior ciliary arteries (6).

B- PULSATILE OCULAR BLOOD FLOW & FUNDUS PULSATION AMPLITUDE

Arterial blood flow to the eye varies with the heart cycle. Blood flow with each pulse causes a change in intraocular volume, primarily via filling of the choroid and, in turn, results in modulation of IOP. Correspondingly, the volume and the IOP are highest during systole and lowest during diastole.

Pulsatile ocular blood flow (POBF) (Figure 1.9) quantifies the pulsatile component of the ocular blood measured during systole which may account for as much as 75% to 85% of the total ocular blood flow (10). Pulsatile blood flow mainly reflects the choroidal circulation with some contribution from retrobulbar pulsations while the role of the retinal circulation is small.

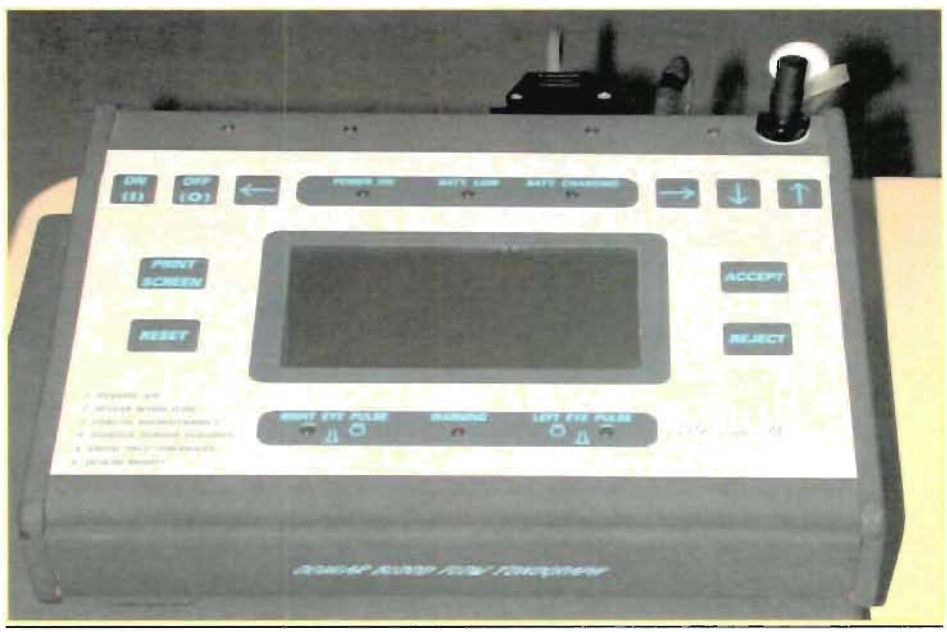

Figure 1.9: Pulsatile ocular blood flowmeter

The Langham POBF technique (11-14) is based on continuous IOP recording which allows measurement of the pulsatile change in IOP during the cardiac cycle. The instrument consists of an applanation pneumotonometer interfaced with a microcomputer that records the ocular pulse. The amplitude of the IOP pulse wave is used to calculate the change in ocular volume and thereby to calculate the pulsatile component of ocular blood flow. Another method for quantification of choroidal

pulsation is by means of interferometry (15,16). A beam of a diode laser (783 nm) is reflected at both the front surface of the cornea and the fundus. The two re-emitted waves produce interference fringes from which the changes in distance between cornea and retina during the cardiac cycle can be calculated. The maximum distance change is called fundus pulsation amplitude (FPA) and corresponds to the pulsatile displacement of the retinal surface caused primarily by the filling of the underlying choroid during systole.

Studies have reported significantly reduced POBF in OAG patients compared to normal subjects and ocular hypertensives (17-19). Reduced POBF was more apparent in high-risk versus low-risk ocular hypertensives (20).

Advantages:

POBF is relatively inexpensive and non invasive, and is simple to perform. Neither clear media nor good fixation is required for accurate measurements. The latest POBF machine is compact, portable and more reliable than its predecessors. FPA gives an estimation of the choroidal pulsations and has good reproducibility.

Limitations:

a- Interpretation of the results derived by POBF is based on a number of assumptions (21): first, the change of IOP is solely caused by volume change due to the blood bolus entering the eye with each pulse; second, retrograde blood flow does not occur; third,

the pressure-volume relationship (ocular rigidity) is standard for all persons; and fourth, the outflow to the venous system is constant and not pulsatile.

b- POBF can only detect the pulsatile component of ocular blood flow and cannot estimate the nonpulsatile component. It is not precisely known what proportions of the total ocular blood flow are pulsatile and nonpulsatile and whether this proportion might vary from eye to eye.

c- The ratio of pulsatile to non-pulsatile blood flow is unlikely to be constant especially with changes of systemic blood pressure or IOP. Changes in pulsatile blood flow are therefore not necessarily representative of changes in total blood flow.

d- POBF measurements were shown to be significantly influenced by factors such as posture (17,18), axial length (22) and heart rate (23). This might have an impact on comparisons between different groups.

e- Measurements of FPA include the displacement of the cornea, thought to contribute as much as 20% of the signal. Like POBF, FPA measures only the pulsatile component of choroidal blood flow.

C- FLUORESCEIN AND INDOCYANINE GREEN ANGIOGRAPHY

In fluorescein fundus angiography, fluorescein is injected into the cubital vein and the passage of the dye is visualized through the ocular vessels (Figure 1.10). Under normal conditions the blood retinal barrier prevents free passage of fluorescein into the tissues. Tight junctions are present both at the endothelial level of the retinal vessels and between the cells of the retinal pigment epithelium.

Fluorescein makes it possible to study the retinal circulation in great detail and the ONH circulation to some extent, while analysis of the choroidal circulation remains more difficult as the endothelium of the choriocapillaris has fenestrations through which fluorescein molecules can pass (24). Indocyanine green (ICG) is thus used for better visualization of the choroidal circulation. ICG is a dye that binds rapidly and completely to certain plasma proteins, preventing the dye from leaving the fenestrated endothelium of the choriocapillaris.

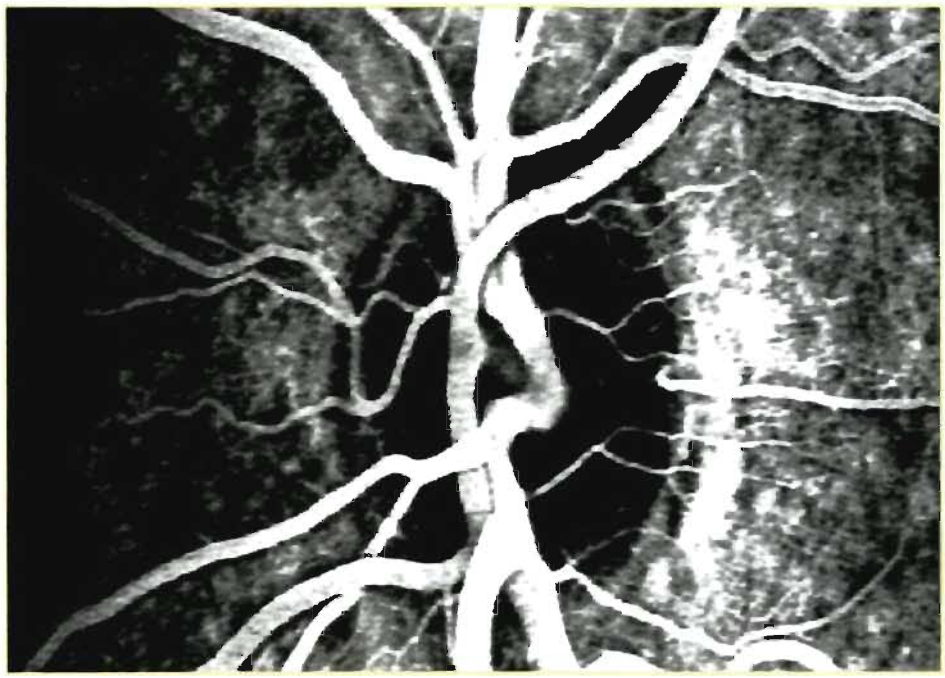

Figure 1.10: Fluorescein fundus angiogram of the optic nerve head and peripapillary retina

Some of the main evidence implicating blood flow deficits in glaucoma is derived from fluorescein and ICG angiography (25-33). In the ONH, filling defects seen on angiography were found to represent areas of ischemia (26,28). Such filling defects

were more marked in glaucomatous discs than in ocular hypertensives and were least marked in normal discs (29). Delayed filling and prolonged arteriovenous passage time have also been described both in the retina and the choroid of glaucoma patients (32,33). The reduction in retinal circulation was reported primarily in OAG patients whereas choroidal blood flow was primarily reduced in NTG patients (33).

Advantages:

Quantification of ONH, peripapillary retinal and choroidal hemodynamics is possible through computerized frame-by-frame analysis of fluorescein or ICG angiograms, (34,35). Confocal imaging techniques help measure filling rates and areas of fluorescein filling defects (34).

Limitations:

a- Inadequate quality of fluorescein or ICG angiograms to outline circulation in the ONH and choroid as well as inadequate resolution to visualize disc vessels arranged in multiple superimposed layers.

b- Reliability of a single angiographic examination in providing information about the ocular circulation.

c- Difficulty in outlining the deeper ONH capillaries. Capillaries of PCA origin are completely masked by capillaries of retinal origin in the surface nerve fiber layer of the disc.

d- Variation in the sources of optic disc fluorescence. Fluorescence of the disc is essentially caused by retinal vessels with little contribution from the deeper ciliary vessels as well as staining from the adjacent peripapillary choroid (36).

e- Significance of the presence or absence of filling defects. Angiography may fail to demonstrate filling defects in cases of transient ischemia caused by temporary fall in ocular perfusion pressure (37).

f- Transient systemic hypotensive changes demonstrated by some patients following the injection of dye might affect the arteriovenous passage time measurements. These measurements may thus not reflect the subject's true ocular hemodynamics.

D. LASER DOPPLER VELOCIMETRY

The Doppler effect was first described by the Austrian physicist Christian Doppler in 1842. It describes the frequency shift that a sound or light wave undergoes when emitted from an object, which is moving away from or towards an observer. Measurement of red blood cell velocity using this technique was first described in 1972 by Riva and coworkers (38).

In laser Doppler velocimetry (LDV), the laser beam is directed at a specific vessel. The back-scattered light contains two components: light scattered by stationary structures such as the vessel wall and light scattered by red blood cells flowing through this vessel. The interference of these two components leads to an alternating signal at the photodetector. This signal is then subjected to a Fast Fourier transform algorithm to

obtain a Doppler shift frequency spectrum (DSFS) which is found to be proportional to the velocity of the moving red blood cells (39,40).

Laser Doppler velocimetry was found to vary with IOP variations. The return of the red blood cell velocity towards normal after an IOP increase above normal or an IOP decrease below normal was reported and attributed to autoregulatory mechanisms (40).

Advantages:

This method provides noninvasive, fast and quantitative measurement of red blood cell velocity at the center of major vessels of the optic nerve head and retina.

With bidirectional LDV, red blood cells velocity is calibrated in absolute values whereas with unidirectional LDV only relative measurements of velocity are possible.

Limitations:

a- LDV provides data on velocity for a single vessel whereas assessment of blood flow requires taking into account the diameter of the measured vessel.

b- LDV measurements can also be affected by changes in the aggregability of the red blood cells in the measured vessel.

E. LASER DOPPLER FLOWMETRY

In 1992, Riva and coworkers (41) described the single-point laser Doppler flowmetry (LDF).

Unlike velocimetry, the LDF measures blood flow in capillary beds with the laser directed at areas between larger vessels.

The technique is similarly based on the Doppler effect with a laser beam of 160 microns focused on a selected location of the ONH or choroid. The LDF allows continuous measurement over several minutes (Figure 1.11) and the Doppler shift frequency spectrum is then identified by a photodetector.

The analysis yields information about relative speed of the red blood cells in the sampling volume (Vel), relative number of moving red blood cells in the sampling volume (Vol) and from these two parameters blood flow (Flo) through the tissue is calculated.

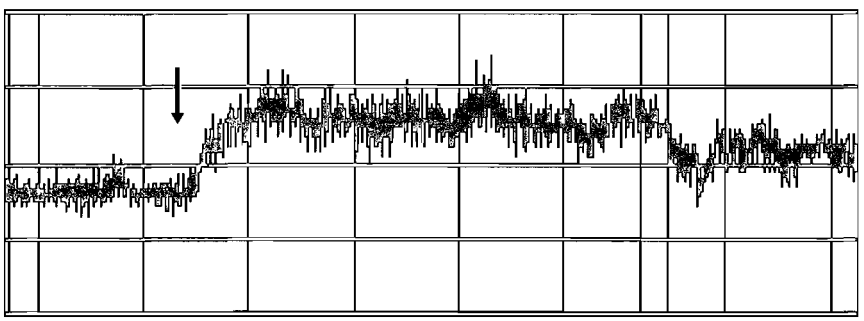

Figure 1.11: Tracing of Laser Doppler Flowmetry for neuroretinal rim blood flow in a normal subject. The arrow marks the onset of a flickering light and the purple line marks the end of the flicker. The recording duration was 80 secs. and Flow is in arbitrary units.

The technique is used to measure microcirculation in the neuroretinal rim or lamina cribrosa. By directing the beam into the fovea (which is not perfused by the inner retinal circulation) sub-foveolar choroidal blood flow can be measured. Using a different laser wavelength, measurements can be also performed in the retina, between the large retinal vessels.

Several physiological studies have been undertaken using the LDF in the ONH (42,43), retina (44) and choroid (45,46). The technique was shown to detect changes in blood flow induced by physiological maneuvers involving breathing of various gases, neuronal stimulation and pharmacological agents. LDF also reported reduced ONH blood flow in glaucoma patients (47) and in glaucoma suspects with no manifest visual field defects (48) compared to control subjects. Lower optic nerve LDF measurements were also reported to correlate well with glaucomatous visual field progression (48,49).

Advantages:

LDF is a powerful technique to investigate intra-individual changes of blood flow at a selected location over a fixed period of time. Vel is expressed in Hz whereas Vol and Flo are expressed in arbitrary units. The technique is highly sensitive, reproducible and has a fast response time. The device is well suited for looking at perfusion changes during one imaging session, i.e. changes provoked by flickering light, inhaled gases, acute pressure changes, or acute drug treatments.

Limitations:

a- The measurements cannot be calibrated and therefore comparisons between individuals are difficult to interpret.

b- Experimental studies using the LDF have reported it having a penetration depth of approximately 400 μm. Therefore in normal optic discs it may measure predominantly the retinal circulation in the surface nerve fiber layer of the disc (6). However in glaucomatous optic discs, where the neuroretinal rim is much thinner, it is more likely to detect a greater proportion of flow values from the deeper SPCA circulation.

c- Measurements are limited to a small selected areas and the exact volume of tissue being measured is unknown. Therefore the technique does not allow easy comparison between individuals and is best-suited for looking at changes from one time-point to another or at circulatory response to a stimulus within the same individual.

F. SCANNING LASER DOPPLER FLOWMETRY

The scanning laser Doppler flowmetry (SLDF) (Heidelberg retinal flowmeter, HRF, Heidelberg Engineering GmbH, Heidelberg, Germany) (Figure 1.12) is a noninvasive instrument combining both a laser Doppler flowmeter with a scanning laser technique. It measures the amount of backscattered light at different locations in the tissue of interest in a short period of time.

Technical features of the SLDF will be discussed in detail in section 5 of this chapter. In brief, an infrared diode laser with wavelength of 780 nm focused at the retina. The laser scans an area of 2.7x 0.7 mm composed of 64 horizontal lines, each with 256 points and

an approximate spatial resolution of 10 μm. Each line is scanned sequentially a total of 128 times with a total acquisition time of 2.05 seconds. A two-dimensional map of microvascular perfusion of the area to be studied is thus generated (50).

SLDF automatic full-field perfusion image analysis (AFFPIA) is a software analysis technique designed to reduce the influence of heterogeneity in the perfusion map, correct for heart beat-associated pulsations as well as enhance the computations generated by the SLDF (51).

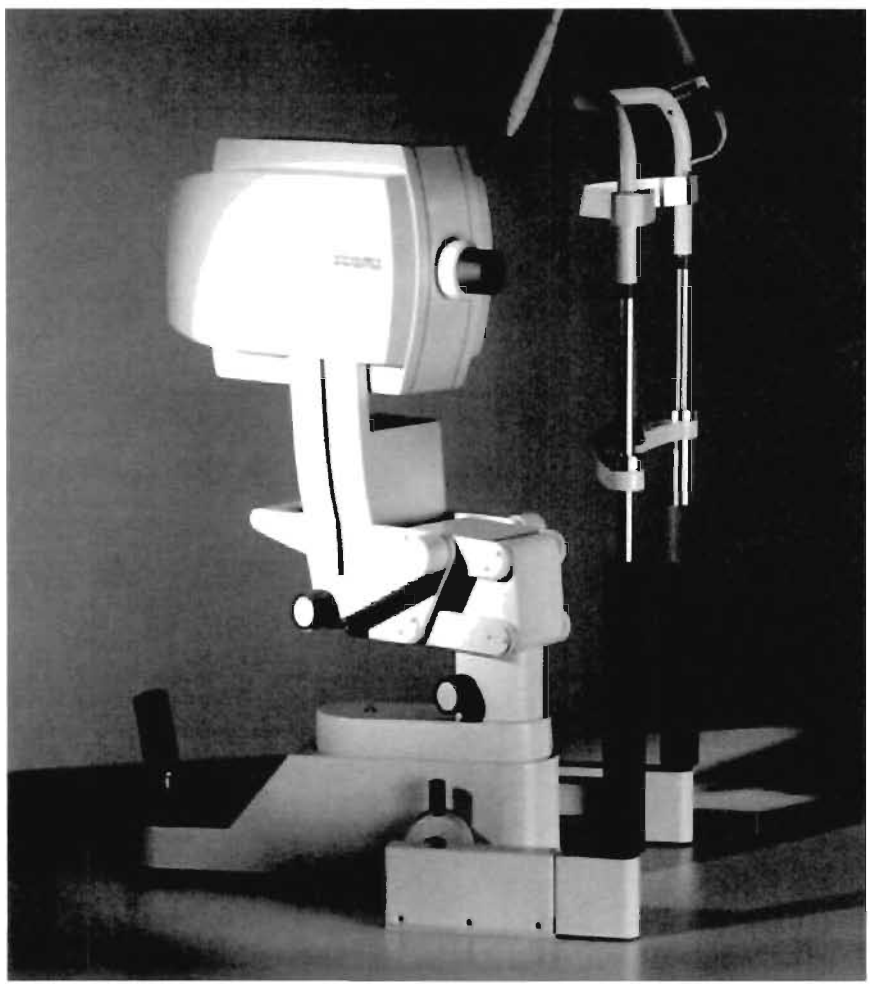

Figure 1.12. Heidelberg Retinal Flowmeter

Advantages:

SLDF is a non-invasive and fast technique. It provides an overall map of retinal and ONH perfusion for a selected location of interest. AFFPIA facilitates the analysis of blood flow in relatively large areas of the neuroretinal rim and peripapillary retina.

Limitations:

a- Total time of image acquisition is 2 seconds and microsaccades during measurement create artifacts by disturbing the Doppler shift signal. Microsaccades can be removed using the AFFPIA software.

b- Clinically insignificant media opacities, particularly posterior nuclear cataracts, degrade the image quality.

c- Uni-ocular patients cannot be tested because fixation with the fellow eye is essential for the 2 second duration of the test.

d- The subjective choice of the area to be measured can be an important source of variation. The slightest difference in the location of the measurement box in relation to retinal or ONH vessels may lead to considerable variation of perfusion values between images. This problem is considerably overcome with the AFFPIA software.

e- The technique is sensitive to illumination changes of the ocular tissue during image acquisition as well as to the low reflectivity of the ONH compared to the peripapillary retina (56).

f- Since there are differences in focusing between the neuroretinal rim and peripapillary retina versus the lamina cribrosa, different focal planes should be used for evaluation of cup and rim perfusion.

g- Accurate analysis of perfusion images is time consuming and requires considerable experience.

E- LASER SPECKLE TECHNIQUE

The technique is based on the principle that a random speckle pattern is created when laser light is focused on a matte surface such as the fundus. The effect is caused by light interference (57) and the image speckle is detected by a sensor. The difference between successive scannings of the image speckles is calculated and integrated for each pixel to obtain the normalized blur, a quantitative index of blood velocity.

Laser speckle flowgraphy demonstrated little change in the superficial ONH circulation following trabeculectomy in a Japanese population of glaucoma patients (58). The technique showed a significant correlation between changes in ONH circulation and visual field damage in NTG but not in OAG (59).

Advantage:

The technique provides non-contact, two-dimensional and valid measurements for retinal and superficial ONH blood flow.

Limitations:

a- The technique measures the superficial blood flow of the ONH. Such measurements reflect the CRA circulation with no contribution from the SPCAs.

b- To date most studies have mainly focused on the validation of the technique and the study of treatment effects.

F- RETINAL VESSEL ANALYSER

The retinal vessel analyzer (RVA; Imedos, Jena, Germany) comprises a fundus camera (FF 450; Carl Zeiss Meditec), a video camera, a real-time monitor and a computer with vessel diameter analysis software. It allows continuous and on-line measurement of the diameter of a segment of a retinal blood vessel with temporal resolution of 25 readings/second (60).

The subject's fixation is adjusted to position the ONH in the centre of the monitor. A square box is then placed by the examiner over the retinal region of interest (ROI) containing the vessel to be analyzed. A rectangular cursor is then placed over the vessel of interest to identify the vessel length (approximately 0.5 mm) to be analyzed for changes in calibre throughout experimentation. The RVA program then initiates analysis of vessel diameter over the length of the vessel within the rectangular cursor (Figure 1.13).

RVA has been shown to have high short term as well as day to day reproducibility with coefficients of variation between 1.3-2.6 % and 4.4-5.2 % respectively (60). The technique demonstrates sensitivity to pharmacologic interventions (62) and reflects changes in vessel caliber consistent with physiological provocation after breathing 100% oxygen (61,62).

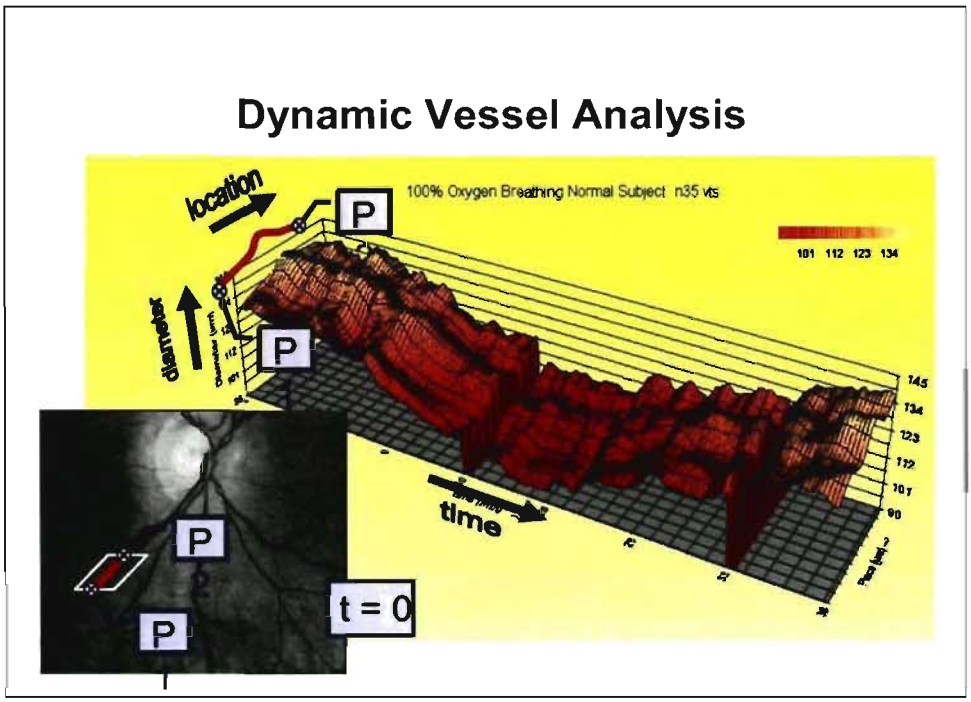

Figure 1.13: Retinal Vessel Analyzer. Fundus image as seen on the device's monitor showing the cursor aligned over the vessel of interest (bottom left) and retinal vessel diameter data collected over 38 seconds (courtesy Dr. Ines Lanzl).

Advantages

a- The technique enables continuous monitoring of the vessel diameter, both as a function of location and time.

b- Using this technique, different vessel segments as well as different retinal vessels can be investigated simultaneously.

c- The system is largely independent of alterations in luminance induced by slight eye movements. Sections of recordings contaminated by eye movements or blinks are automatically eliminated from the analysis.

d- Fundus images are stored on a videotape recorder for off-line measurement of other vessels within the captured field of view.

Limitations:

a- RVA does not allow absolute retinal vessel size measurements which may limit its use in cross sectional studies.

b- While giving a reproducible assessment of vessel diameter, the technique does not evaluate blood velocity.

G- CANON LASER BLOOD FLOWMETER

The Canon laser blood flowmeter (CLBF 100, Canon, Tokyo, Japan) measures the blood column diameter, blood velocity and blood flow in major retinal vessels.

The technique is based on the principle of bidirectional laser Doppler velocimetry (63). It utilizes a fundus camera and two low intensity lasers, one for measuring retinal artery blood velocity and the other for measuring vessel diameter. A beam from a red 675-nm diode laser is used for velocity measurement and is emitted from a fundus camera measuring head. The Doppler-shifted light scattered from the flowing blood cells in the target vessel is detected simultaneously in two directions separated by a fixed angle. The signals from the two tube detectors undergo computer-controlled spectrum analysis and sequential measurements of velocity are performed automatically. Results are acquired at 50 measurements per second for 2 seconds. A stripe provided by a green 543-nm HeNe laser oriented perpendicular to the target vessel is used to measure the diameter of the retinal vessel. The diameter is determined by computer analysis of the

captured image of the vessel. From these two measurements, flow is accurately calculated by the instrument's software in actual units of µl/min (64) (Figure 1.14).

The CLBF is equipped with an internal fixation target, a vessel tracking system and a pupil centration device. These elements help to monitor the target vessel and maintain centration of the laser beam at all times during measurement and thus improve the accuracy of the technique.

CLBF measurements have been shown to be valid as well as reproducible (65,66). Mean intersession coefficients of variability for the technique have been reported to range between 16.4% (65) and 19.3% (66) for flow in vessels of normal subjects.The technique is well suited for evaluating changes in retinal blood flow during or between sessions. However, comparisons between retinal vessels in OAG patients and OHT patients or normal subjects have not been reported to date.

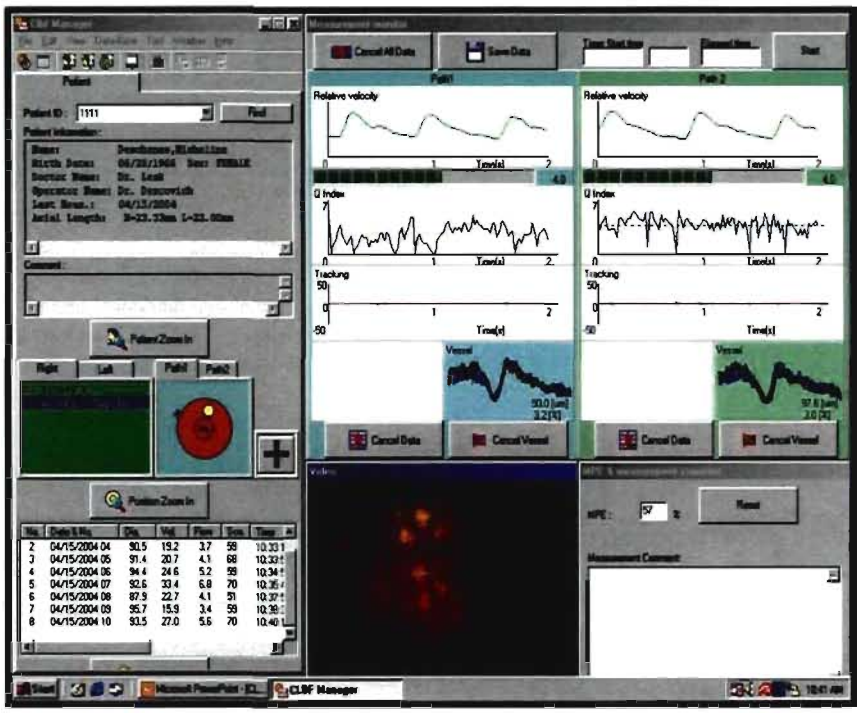

Figure 1.14: CLBF measurement of the inferior temporal retinal artery showing the tracking stripe (fundus image, bottom center). The display shows a plot of the velocity variation during the cardiac cycle (green line, top centre and top right; plot is over two seconds) and the vessel diameter (V-shaped plots, center and right) as well as a tabulation of the data analysis with values for blood column diameter, centre-line blood velocity and blood flow from successive measurements (table, bottom left).

Advantages:

a- Vessel diameter measurements are corrected for the axial length of the eye (operator input) and refractive error of the eye (measured by the CLBF).

b- The position of the measurement is recorded on the captured fundus image for subsequent comparisons.

c- Only Doppler curves with a high quality "Q-index", as determined by the instrument's software are accepted.

d. Because it measures both vessel diameter and blood velocity, the CLBF is the only existing technique that gives quantitative measurements of retinal blood flow.

Limitations:

a- Comparisons between populations may be difficult to make because of the inter-subject variation in the location of measurement in relation to the vascular tree.

b- When the targeted vessel segment is very close to another vessel, the tracking device sometimes skips to the other vessel.

H- BLUE FIELD ENTOPTICS

The technique is based on the flow of white blood cells to assess macular blood flow. Subjects can perceive the presence of leukocytes in the capillaries around the macula when looking at diffuse blue light of 430 nm. Similar patterns are created by computer simulation and subjects are then asked to match the speed of the computer-generated particles with those that they see in the blue field. The pattern match can then be used to draw conclusions about perifoveal perfusion. The method was developed by Riva and Petrig (67) for subjective evaluation of perimacular leukocyte velocity and density.

Using blue field entoptics, studies reported abnormal autoregulation of macular blood flow in OAG (68) as well as a significant positive correlation between loss of visual function and reduced leukocyte velocity (69).

Limitations:

a- The technique is based on the assumption that macular capillaries have a fixed diameter

b- The patient must estimate the number of moving white blood cells. However, the accuracy of such estimations may be affected by the physiologic and pathophysiologic state of the retina.

c- The conclusions that can be drawn from the technique are limited as the quality of the data depends on the patient's cooperation and perception.

d- Large variations between patients exist and only data from perifoveal capillaries are provided.

I- PERIPHERAL BLOOD FLOW

Blood flow disturbances in glaucoma patients point to some correlation between ocular blood flow and peripheral blood flow. Since vasospasticity and vascular dysregulation have been linked to glaucoma we will briefly review the methods of assessment of peripheral blood flow.

Evaluation of peripheral blood flow has been performed using two methods: microscopy of the nailfold capillaries of the fingers (70) and LDF measurement of bulk blood flow at selected locations (71).

Using microscopic examination of the cellular elements in the nail-fold capillaries, blood flow velocity can be monitored and videotaped for analysis. A provocation test

with local cold exposure of the nail-fold area to cold air (–15°C for 60 seconds) results in a significant reduction of the velocity of erythrocytes in patients with vasospasm. The reduction can be so marked that a cessation of blood flow in the nail-fold capillaries can be observed. Digital vasospasm is defined as a closing of one or more visible capillaries, with a mean stoppage time of longer than 12 seconds.

Laser Doppler flowmetry (LDF, Transonic Systems Inc., Ithaca, NY) is another technique that uses a low intensity laser beam to illuminate the nailfold capillaries of the finger. A receiver detects light reflected by both stationary structures and moving particles (mainly red blood cells). The latter portion of reflected light undergoes a Doppler frequency shift allowing the computation of blood flow. The methodology used for LDF finger blood flow measurements as well as the definition of a positive vasospastic response were both based on a leading study by Drance et al (72). Baseline flow was measured on the underside of the end of the middle finger of a randomly selected hand. After a stable flow reading was obtained the hand was immersed in warm water (40°C) for 2 minutes. The hand was then immersed in ice-cold water (4°C) for 10 seconds and then finally placed at room temperature for 10 minutes (recovery period) (Figure 1.15).

Finger flow measurements were made continuously by the laser Doppler flowmeter and transmitted in real-time to a computer via an interface. Drance defined a ratio of maximum to minimum flow of more than 7 as vasospastic, but low baseline flow and low flow upon cold exposure may also be signs of vasospasticity.

Studies of peripheral blood flow have suggested the possible role of vasospastic phenomena in the development and progression of glaucomatous optic neuropathy. Blood flow in both the nailfold capillaries of the fingers (73) and the microcirculation of the skin (74) was reported to be reduced in glaucoma patients.

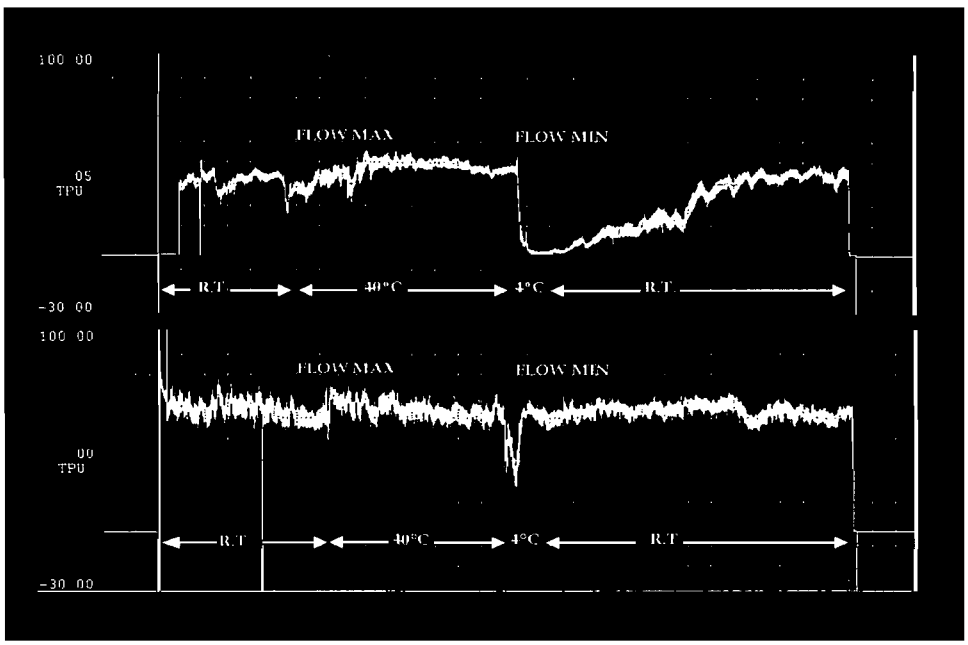

Figure 1.15: Top: Tracing of Laser Doppler flowmetery (LDF) in a vasospastic patient showing a low baseline flow at room temperature and a marked decrease in flow after immersion of the hand in cold water (4°C) with a delayed recovery to baseline. Bottom: Tracing of peripheral blood flow in a nonvasospastic patient showing a normal baseline flow at room temperature and a rapid decrease after immersion of the hand in cold water (4°C) with a rapid recovery to baseline.

Advantages:

Patients do not need to fixate or have clear media.

Limitations:

Finger LDF measurements are sensitive to movement by the patient and distractions such as talking, so measurements have to be performed under controlled conditions.

J- ANIMAL EXPERIMENTAL METHODS

Most of these methods are invasive and are briefly mentioned to give the reader a full picture of the field of measurement of ocular blood flow.

1. Oxygen tension method: based on measurement of intra-vascular pO2 using a phosphorescence imaging technique as well as measurement of extra-vascular pO2 using microelectrodes placed in front of or within the ONH (75)
2. Microspheres method: unlabelled or radioactive labeled or nonradioactive colored microspheres have been used to study ocular and ONH blood flow in animals.
3. Autoradiographic methods using iodoantipyrine: the method has been used in animals to evaluate blood flow in the retina, choroid and ONH (76,77) as well as to demonstrate autoregulation in the ONH and lamina cribrosa.
4. Labile liposomes method: encapsulated heat-sensitive liposomes containing fluorescent dye have been injected intravenously and lysed by a heat pulse delivered by laser (78).
5. Hydrogen clearance method: optic disc blood flow has been measured in rhesus monkeys by inserting a microelectrode in the lamina cribrosa and determining the half-time for clearance of hydrogen gas from the saturated tissue (79).

6. Krypton washout method: used to measure blood flow in the retinal vessels and in the choriocapillaris but not in the ONH (80).
7. An innovative 40MHz ultrasound biomicroscope pulsed Doppler probe can measure blood flow in various ocular tissues but has a depth of penetration that is suitable for rodent not human eyes (81).

Limitations of Ocular Blood Flow Assessment Techniques and their Interpretations:

The difficulties involved in the assessment of ocular blood flow have led to a variety of innovative techniques. These techniques have been used in both experimental animal models and human clinical investigations. While each technique may provide valuable information about the status of a particular vascular bed within the eye, each also has its limitations and restrictions that must be taken into account when interpreting their measurements.

Many of the techniques available measure either velocity of blood cells or transit times for fluorescein. These measurements cannot be translated directly into flow measurements, unless the precise diameters of the blood vessels measured or information about the total volume of the vascular bed is known. Other techniques examine physiological parameters such as the oxygen profile in the retina. These are not direct measurements of blood flow but rather estimates of alterations in oxygen delivery

and metabolic demands and have proven useful for studying the effects of increased IOP on the retina and ONH.

Fluorescein angiography (FA) remains the basic technique for studies of retinal blood flow in clinical practice. In its basic form FA provides qualitative information though recent attempts have been made to obtain quantitative information as well. Major limitations still exist in quantitative interpretation. Both color Doppler imaging and ocular pulse amplitude provide useful information. However, it is important to understand what aspect of ocular hemodynamics each method measures so that their relevance in glaucoma is understood. CDI provides its most reproducible measurements for the ophthalmic artery and CRA and the resistivity index is calculated from the velocities measured. Information provided from the SPCAs, the most important source for ONH perfusion, is significantly less reproducible. Ocular pulse amplitude is a measure of the pulsatile component of choroidal blood flow, which does not correlate necessarily with total blood flow and which is a poor estimate of blood flow in other ocular tissues.

The principle of frequency shift induced by moving objects (Doppler shift) has been widely used in the estimation of ocular blood flow in the ONH, retina and choroid. These techniques have been arguably most successful when applied to the retinal arteries where both velocity and diameter can be measured with reasonable accuracy. When applied to the ONH, the techniques of laser Doppler flowmetry and SLDF remain sensitive to blood flow changes in the superficial layers of the ONH with an estimated

depth of penetration of 400 microns. A highly accurate and reproducible technique for the measurement of blood flow in the pre-laminar and laminar ONH remains elusive.

Ocular blood flow techniques will continue to evolve. There are recent and promising advances in Doppler ultrasound and spectral domain OCT coupled with Doppler technology. Several research groups are making advances in fundus spectroscopy. Detection of metabolic byproducts, the consequences of relative ischemia, may soon be realizable. Most of these advances are currently research tools with too little known to assess which ones will find a practical clinical application. The prospects for the continued evolution of such technologies and for the knowledge obtained from their application is promising although successful use will always depend on a thorough understanding of the limitations of the data obtained.

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TECHNICAL ASPECTS OF SCANNING LASER DOPPLER FLOWMETRY AND SCANNING LASER OPHTHALMOSCOPY

Evaluation of the morphologic and perfusion changes in the glaucomatous ONH requires an accurate assessment of the disc appearance and its blood flow both at baseline and in response to sustained IOP reduction. Recent technological advances have enabled the study of such changes noninvasively and directly in the ONH. Both scanning laser Doppler flowmetry and confocal scanning laser ophthalmoscopy have been shown to provide valid, accurate and reliable measurement of the ONH topography and blood flow.

A. Scanning Laser Doppler Flowmetry

The scanning laser Doppler flowmetry (SLDF) also referred to as Heidelberg retinal flowmeter (HRF, Heidelberg Engineering GmbH, Heidelberg, Germany) is a scanning laser Doppler flowmeter (SLDF) used to visualize the microvasculature and measure the perfusion of the ONH and retina.

It is a non-invasive instrument combining both a laser Doppler flowmeter with a scanning laser technique to estimate ONH and retinal capillary perfusion. The imaging system contains a scanning beam and so hemodynamic parameters at discrete locations

can be obtained. The imaging system is also confocal and thus signals from axial planes other than the focal plane of the laser are suppressed (1-5).

Principle

The SLDF records blood flow in vascularized tissues based on the optical Doppler effect. The Doppler effect is caused by a frequency shift of waves reflected by moving objects.

This is based on the equation: ($\Delta f/f \sim v/c$) where "Δf" is the Doppler-shifted frequency, "f" is the original frequency, "v" is the velocity of the object and "c" is the velocity of light (Figure 1.16). Hence, by measuring the Doppler frequency shift, it is possible to estimate the velocity of the object in question (6).

The combination of the Doppler-shifted light that comes from moving red blood cells "$f + \Delta f$" and the unshifted portion that comes from non-moving structures such as vessel wall and tissues "f" produces an interference (Figure 1.17).

The interference of these two reflected frequencies creates a repetitive oscillation of intensity or a "beat". The resulting wave has a frequency of "$f + \Delta f/2$" while the beat frequency is Δf. A photodiode detector detects this beat in the form of intensity variations of reflected light and allows computation of the Doppler frequency shift (7).

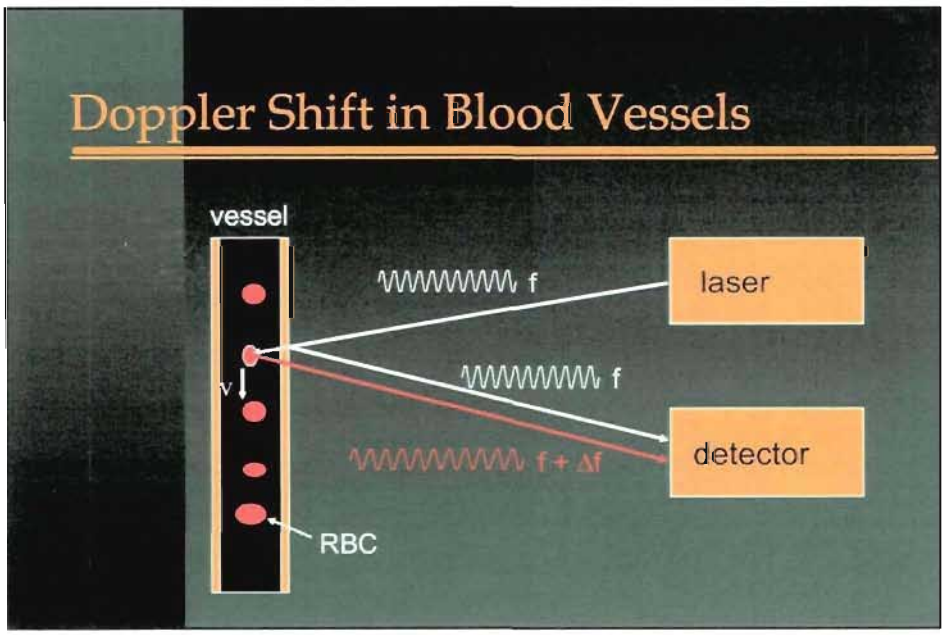


Figure 1.16: The Doppler effect is caused by a frequency shift of waves reflected by moving red blood cells (RBC). "f" is the original frequency "Δf" is the Doppler-shifted frequency.

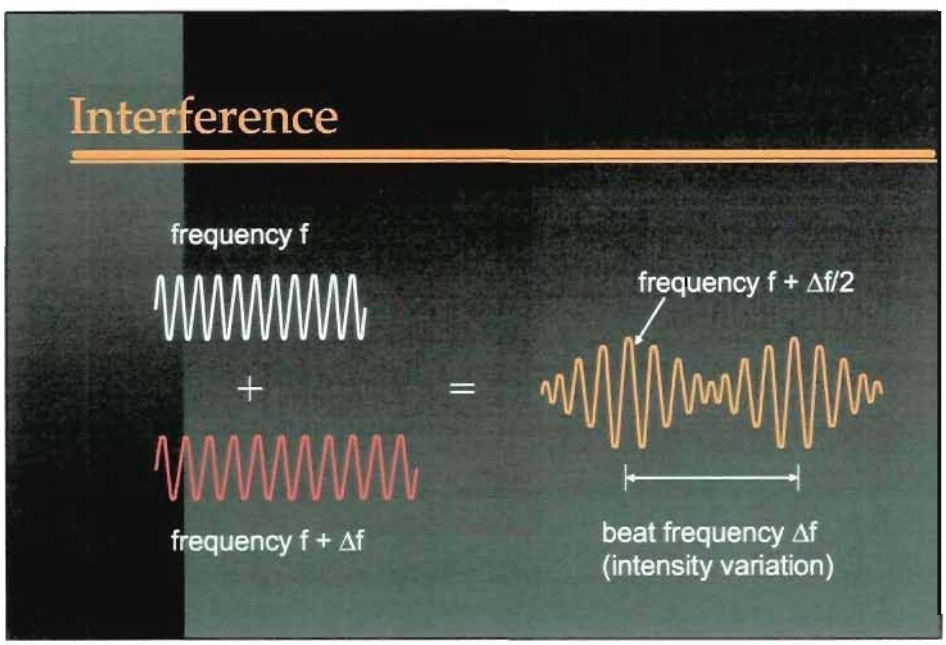


Figure 1.17: The interference of the two reflected frequencies creates a repetitive oscillation of light intensity or a "beat". This oscillation has a frequency of "f+Δf/2" and is proportional to the Doppler shift Δf.

Intensity variations are then subjected to a Fast Fourier Transform (FFT) to convert the intensity values (as a function of time) to a power spectrum (as a function of frequency) (Figure 1.18) (2,3,5).

The treatment of this data in order to determine the perfusion parameters of volume, flow and velocity has been described in 1981 by Bonner and Nossal (8,9):

1. "Volume" is proportional to the number of red blood cells moving in the sample tissue
2. "Flow" is proportional to the distance traversed by all moving cells inside the sample tissue per unit time.
3. Mean blood cell speed (Velocity) is then determined from the quotient (Flow/Volume).

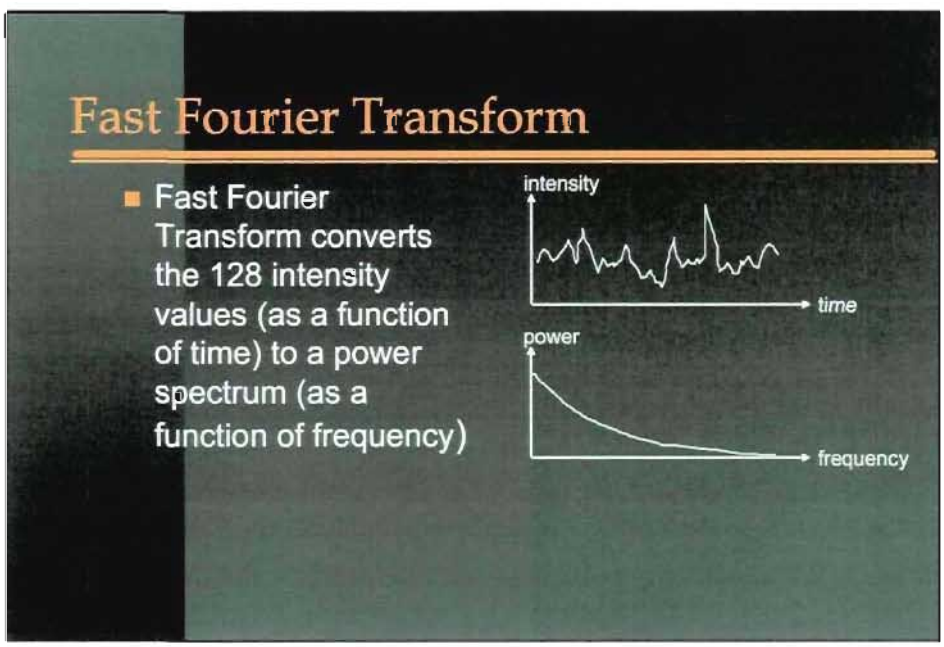


Figure 1.18: Fast Fourier Transform converts the intensity values (as a function of time) to a power spectrum (as a function of frequency).

Application of the Bonner and Nossal theory requires that the Doppler-shifted light be scattered randomly from different directions prior to detection. The microcirculation of the ONH appears to meet this condition, with capillaries oriented in different directions. This is in contrast to larger retinal vessels, where a specific uniform orientation and a single direction of flow can be determined and thus the angle between the incident laser light and backscattered light is known (10). The flow velocities of such vessels cannot, therefore, be correctly assessed using this technique.

Instrumentation

An infrared diode laser with wavelength of 780 nm and a sampling frequency of 4,000 Hz, permitting the detection of Doppler shifts with frequency up to 2,000 Hz is utilized. The laser is focused on the retina and scans an area of 2.7 mm x 0.7 mm. This area is composed of 64 horizontal lines, each with 256 points and approximate spatial resolution of 10 μm. Each line is scanned sequentially a total of 128 times with a total acquisition time of 2.05 seconds.

The result is a 2-dimensional map of microvascular perfusion of the area to be studied from which the parameters volume, flow and velocity are calculated in arbitrary units (AU).

Analysis

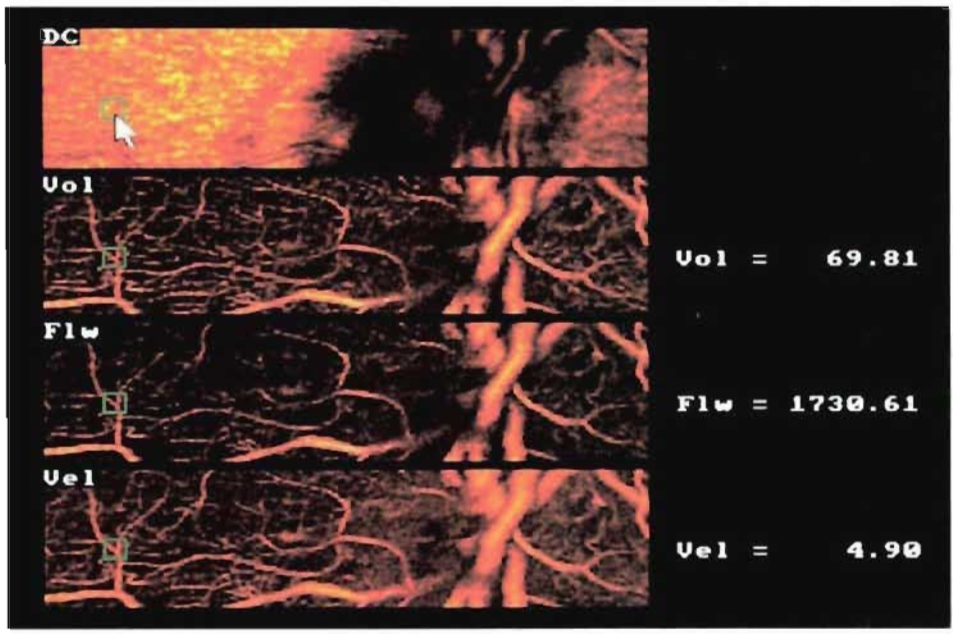
When first introduced, HRF permitted analysis by placement of a measurement box of 10 x 10 pixels in the region of interest within the scan area (Figure 1.19 A). Using this method was reported to show several technical limitations including (11):

- Artifacts caused by saccades (eye movement artefacts of approximately 30 msec or larger) occurring during the two seconds of image acquisition disturb the Doppler shift signal.
- Subjective variation in the position of the measurement box selected by the operator between images.
- Placement of the box on the neuroretinal rim of glaucoma patients with high C/D ratio and thin rim unavoidably includes an area of the cup or the peripapillary retina in the box thus leading to erroneous data.
- Appropriate illumination of the ocular tissue during image acquisition taking into consideration the low reflectivity of the ONH compared to the peripapillary retina and the influence of brightness on the measurements

In an attempt to improve the reproducibility of the HRF, investigators proposed various methods for analysis of perfusion data. Kagemann et al (12) used flow histograms and pixel-by-pixel analysis of the entire perfusion map. Hosking et al (13) introduced a search strategy in which a 10x10 pixels box was repositioned within a 15x15 window and 36 mean flow values were extracted whereas Sehi et al (14) proposed to exclude the upper and lower 25% of flow values within the 10x10 pixels measurement box aiming to reduce outliers.

SLDF automatic full-field perfusion image analysis (AFFPIA) is a software designed to reduce the influence of heterogeneity in the perfusion map, correct for heart beat-associated pulsations as well as enhance the computations generated by the HRF (15). The software eliminates pixels with incorrect brightness as well as marks saccades that lead to erroneous perfusion data. It also excludes pixels of retinal vessels with a diameter greater than 30 μm, whose flow velocities are too high for the sampling frequency afforded by this technique. The analysis is based on the average of all valid pixels in the entire scan area rather than data obtained from a discrete target square (Figure 1.19 B).

The potential effective measuring depth into a tissue using the SLDF has been estimated at between 300 and 400 microns when the laser is focused on the tissue surface. Wang et al (16) estimated using microspheres the depth of penetration of scanning laser Doppler flowmetry in the anterior optic nerve of monkeys. They concluded that when focused on the surface of the neuroretinal rim of the ONH, SLDF measures perfusion mainly from the superficial ONH where the vascular supply is primarily from the CRA. However in glaucomatous optic discs, since the neuroretinal rim layers are much thinner, it is more likely to detect a greater proportion of flow values from the deeper SPCA circulation.



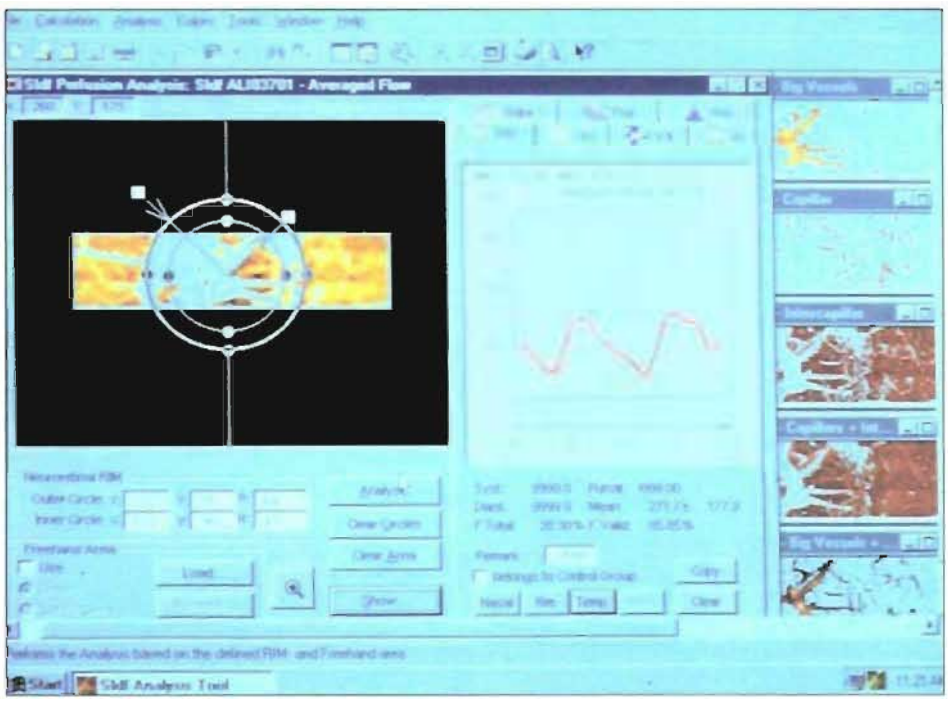


Figure 1.19: A. SLDF perfusion map showing the standard 10 x10 pixel measurement box and the relative values for volume, flow and velocity (Top). B. SLDF full-field perfusion image analysis of the neuroretinal rim area after exclusion of vessels with a diameter of >30 μm and outlining of the ONH and the neuroretinal rim (Bottom).

Validity and Reproducibility

Several authors have investigated the validity of the SLDF for quantitative evaluation of retinal and ONH perfusion.

Michelson and Schmauss (2) reported a significant linear relationship between SLDF flow and ocular perfusion pressure while varying the IOP by a suction cup in normal volunteers. Furthermore, the authors compared measurements of corresponding retinal points by SLDF and single-point laser Doppler flowmeter and reported a significant and linear relationship in normal and glaucomatous eyes (3).

Using a model flow system of glass capillaries coupled to a syringe and perfused with skim milk over a range of pump flow rates, Chauhan and Smith (10) reported a high significant linear correlation between the SLDF-measured flow and actual flow within a given operating range. A significant linear correlation was similarly reported by Tsang and colleagues (17) using a similar model with both light and dark backgrounds.

Other investigators (18,19) have also shown that the SLDF is appropriate for description of the effect of graded changes in blood gases on retinal hemodynamics. They noted that changes in measured blood flow at the ONH occurred in the expected direction in response to blood gas perturbations.

The reproducibility of SLDF measurements has also been demonstrated within a certain range. The technical design and anatomy of ocular blood flow has imposed some limitations on the technology. Higher variations can reduce the power to detect statistical differences between groups or monitor manipulations of ONH blood flow.

Using the 10x10 pixel measurement box, reported intrasession reliability for flow in a retinal location of normal subjects was 0.84 (2) while intersession reliability ranged between 0.62 and 0.82 (3). However, in a rim location, the reported intersession reliability was 0.47 in normal subjects and 0.36 in glaucoma patients (20). In one study, using flow histograms and pixel by pixel analysis of the entire perfusion image, the intersession coefficient of variation for flow decreased from 30.1% to 16.3% (12).

Using AFFPIA, Michelson and associates reported intersession reliability of 0.74 for flow in the retina in normal subjects (15). This was in the same range as the reliability of the analysis of the measurement box used in the original HRF software. However, Michelson and associates used only one image and consequently one measurement per session for their computations. In another study, Iester et al (21), using the same technique, reported a coefficient of variation between 20.3% and 24.4%. However, no reproducibility data was reported using AFFPIA when perfusion values were obtained from the mean of multiple perfusion images.

Technical Limitations

- SLDF perfusion parameters are reported in arbitrary units as they are normalized relative to the "DC" (Direct Current) value (22). DC value refers to the power spectrum for a location that has a frequency of zero. This makes the comparison between HRF measurements and other OBF techniques difficult.
- HRF has experimentally demonstrated measurable flow values when there is no actual flow (10). This zero-offset is related to the intrinsic noise and random

variation in the intensity of the back-scattered light that is not related to Doppler shift

- Clinically insignificant media opacities degrade the quality of the images as well as increase the underlying noise and thus artifactually increase the overall measured perfusion values.

B. Confocal Scanning Laser Ophthalmoscopy

The confocal scanning laser ophthalmoscope (CSLO) also referred to as the Heidelberg Retinal Tomograph (HRT, Heidelberg Engineering, GmbH, Heidelberg, Germany) is a confocal scanning laser system that acquires three dimensional high-resolution images of the posterior pole (23,24). It allows quantification of the ONH and follow-up of topographical changes. The technique is noninvasive, rapid and reproducible.

Principle

The HRT employs a scanning ophthalmoscope with an infrared diode laser and a confocal optical system for image acquisition.

This system contains a pinhole situated in front of a detector, which measures the intensity of the light reflected from the fundus. The pinhole ensures that the detector captures the light reflected from the focal plane of the laser and eliminates light reflected from locations in front of or behind the focal plane. Scanning mirrors move the deflected laser beam on a 2-dimensional plane perpendicular to the optical axis. The resulting 2-dimensional image is an optical section of a 3-dimensional section. Varying the depth of the focal plane allows acquisition of cross-sectional images of the ONH from other focal planes along the z-axis.

Instrumentation

Using a diode laser of wavelength 670 nm and a spot size of approximately 10 microns, the HRT acquires a series of 32 optical sections at consecutive focal planes. Each image

consists of 256 pixels x 256 pixels, each pixel corresponding to the ONH or retinal height at that location. For each pixel, reflected light intensities are measured at different focal planes in different depth locations. The depth of each topographic image series ranges from 0.5 mm to 4.0 mm depending on ONH morphology.

Both scan area and scan depth can be adjusted by the operator depending on the size of the optic disc and the depth of the optic cup. Total image acquisition time is approximately 1.6 seconds.

Because of the long acquisition time and the high resolution of the device, images must be aligned to correct for small eye movements that occur during scanning. This alignment procedure ensures that each pixel location in all images of the optical section correspond to the same location on the fundus.

For each pixel, reflected light intensities are measured at different focal planes in different depths. The distribution of these light intensities along the optical axis z is called "intensity or z-profile". This z profile has a symmetrical shape with the highest intensity at the location of the light reflecting surface whereas the intensity drops rapidly with increasing distance from that surface. By determining the position of the profile's maximum, it is possible to determine the location of the light reflecting surface. When the same calculation is performed at all pixels of the image, the result is a matrix of 65,536 (256 pixel x 256 pixel) independent height measurements in a field

of view of 10°. The matrix can be visualized as a color-coded or a grey-scale topography image.

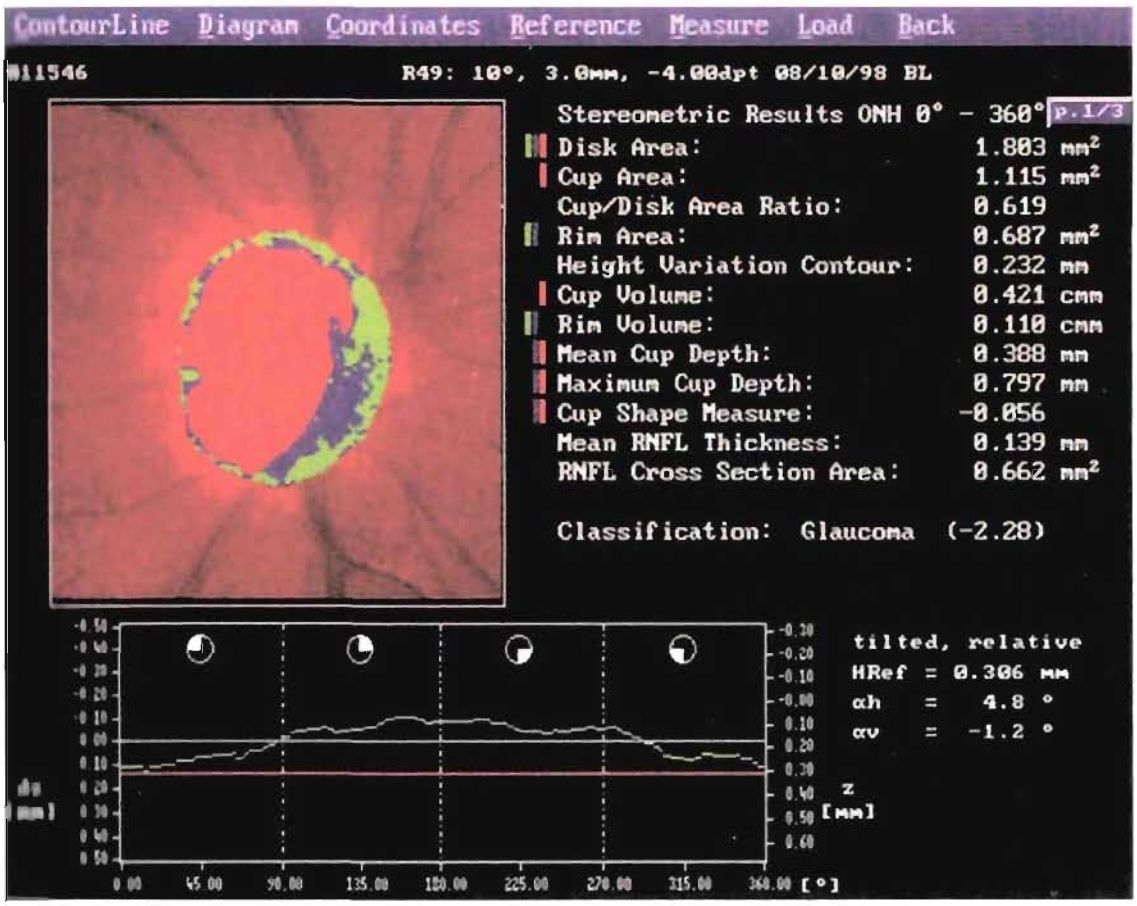


Figure 1.20: HRT analysis showing stereometric parameters of the ONH (upper right) and topographic image (upper left). Red area within the disc delineates the cup, green area the rim and blue area the slope). Green line (below) shows the circumferential height of the contour line and red line the reference plane height.

Analysis

The operator then manually defines the border of the optic disc (contour line) on the topography image (either a single scan or a mean of several scans). This contour line is stored and then exported to subsequent images of the same eye. A standard reference

plane based on the height of the contour line is then determined. The default position of the reference plane lies 50 microns below the average height of the contour line in an inferior temporal segment between 350 degrees and 356 degrees. This represents the part of the papillomacular bundle probably affected last in glaucoma and therefore the most stable position for a reference plane.

Calculated parameters are given in absolute units and include cup area, cup/disc ratio, rim area, mean cup depth, cup volume, rim volume, maximum cup depth, cup shape measure, height variation contour, retinal nerve fiber layer thickness and surface area (Figure 1.20).

Detecting progression of HRT parameters depends on the ability to observe a change in topography over time. Several statistical approaches have been developed for that purpose. All require using the first image as baseline exam and subsequent images as follow up exams, importing the contour line defined at baseline to follow up images and then comparing parameters from the two exams.

Validity and Reproducibility

HRT has been reported to differentiate between ONH of normal individuals and glaucoma patients with sensitivities ranging from 62%-87% and specificities ranging from 73%-94% (25-27). However, the value of the technique as a one–time definitive test for diagnosis of glaucoma is still uncertain. Agreement between HRT parameters and conventional techniques for ONH assessment (e.g. stereo photos) have been

generally conflicting, with studies showing HRT measurements of the neuroretinal rim to be larger (28,29), smaller (30,31) or identical (32).

Detecting progression of glaucomatous damage represents the most significant use of HRT. In contrast to stereo photos, HRT can detect alterations in ONH topography following therapeutic IOP reduction (33-35). Numerous studies have also shown that HRT topographic parameters, particularly cup shape measure, correlate well with visual field defects (36-40).

Variability of topographic parameters has also been studied extensively by several authors. Using an earlier version of the HRT, the laser tomographic scanner, Dreher et al (41) reported standard deviations of 49.4 microns in glaucoma patients and 42.6 microns in normal subjects with no statistically significant difference in variability of height measurements between the two groups.

Enhancement of image quality was possible through more rapid image acquisition, higher signal to noise ratio and longer illuminating wavelength. This improved the standard deviation of single-pixel height measurements to 32 and 30 microns in glaucoma patients and normal subjects respectively (42). Similar values were reported by Weinreb et al (43) (40 microns for glaucoma patients and 35 microns for normal subjects) and Chauhan et al (44) using 16-fold condensed topographic maps (31 microns for glaucoma patients and 26 microns for normal subjects).

HRT coefficient of variation was reported to be around 10% (45) with measurements of disc and neuroretinal rim showing less variation compared to parameters such as height variation in contour and cup shape measure. Several studies (25,44,46) reported consistently higher ONH coefficients of variation in glaucoma patients compared to normal subjects, which supports the idea of a relationship between severity of damage and variability. HRT variability increases with age (44), steep topographic contours at the edge of the cup and along vessels (44,47), media opacities (48) and decreased pupil size after pilocarpine treatment (48). HRT variability is also affected by cardiac cycle (49).

Variability contains elements due to technical variables as well as elements due to physiologic changes. Studies have shown that the variations in topographic measurements in a model eye were lower than in human subjects (50) which suggests that physiological changes as ocular pulse and alignment are the major source of variations. Operator skill can also influence the variability of the technique since the operator controls factors as scan depth, focus and image alignment (51,52).

Short-term and long-term variability components of HRT parameters in normals were shown not to be different, suggesting that variability was not influenced by images obtained at different sessions (53)

Technical Limitations

- Absence of clear consensus on what changes are clinically relevant in both topographic parameters or probability maps.

- Parts of the central retinal vessel trunk within the contour line and outside the cup are sometimes included in the measurement of the neuroretinal rim by the HRT algorithm when there is no nerve fiber tissue beneath the vessel. This leads to errors in the estimation of rim area and width (28).
- Reflectance properties from peripapillary temporal crescents or chorioretinal atrophic changes can provide erroneous topographical measurements and confound interpretations.
- Pupillary dilatation is sometimes necessary to obtain good quality images especially with patients on miotics (pupils < 3mm) or in the presence of cataracts.

CSLO Upgrades

HRT upgrades are intended for use in the clinical milieu by various operators, some of whom may not possess the level of experience required for the successful use of the previous model.

HRT II, introduced in 1999, demonstrates a resolution of 384 x 384 pixels over a scan area of 15 degrees. A total of 16 equally-spaced confocal image sections per 1 mm of scan depth are acquired. Other features include automatic assessment of scan depth and acquisition of multiple sets of confocal images to compute a mean topography.

The software uses Moorfields Regression Analysis for early glaucoma detection showing the ratio of rim area to disc area in six sectors. It also allows for progression detection after normalization of both the baseline and the follow-up visits to each other.

Local change between the surface heights of the two measurements in microns is identified and color-coded. The images are then further analyzed using an array of 4x4 pixels called "superpixels" creating a topographic change analysis.

HRT 3, introduced in 2005, provides all the advantages of HRT II. It addition, it does not require the plotting of a contour line or utilize a reference plane.

The software uses both Moorfields Regression Analysis and Glaucoma Probability Score for early glaucoma detection. The software includes expanded and ethnic-selectable normative data-base and all parameters are adjusted for disc size. Progression detection is similarly done by topographic change analysis using computations for both area and volume of change.

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Chapter 2

OBJECTIVES AND STUDY DESIGN

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OBJECTIVES

A. PRINCIPAL RESEARCH OBJECTIVE AND HYPOTHESIS:

To evaluate the vascular and morphologic changes of the ONH and peripapillary retina following therapeutic intraocular pressure reduction in OAG and OHT. The presence and extent of blood flow changes in the ONH and peripapillary retina will be correlated with ONH morphologic changes as well as peripheral vasospasm and central corneal thickness. A correlation between ONH changes and long term visual field stability will also be studied.

Hypothesis:

Following therapeutic IOP reduction, OAG patients will demonstrate improvement in both ONH blood flow and ONH morphology when compared to ocular hypertensives. Such improvement will correlate with peripheral vasospasm and with central corneal thickness. Such changes will also be prospectively associated with the development of long-term visual field progression.

B. SPECIFIC RESEARCH OBJECTIVES AND HYPOTHESIS:

1. To evaluate the reproducibility of the SLDF full-field perfusion image analysis of the neuroretinal rim and peripapillary retina in a population of OAG and OHT patients as well as in normal volunteers. Also to determine the optimal number of measurements needed to obtain highly reproducible perfusion values.

   Hypothesis: SLDF full-field perfusion image analysis will be more reproducible than the original Heidelberg Retinal Flowmetry (HRF) software using 10x10 pixels

windows. Reproducibility will improve when data is derived from multiple successive perfusion images as opposed to a single image.

2. To compare blood flow in the neuroretinal rim of the ONH and in the peripapillary retina using SLDF full-field perfusion image analysis in OAG patients, ocular hypertensives and normal subjects.

   Hypothesis: OAG patients will have lower ONH and retinal perfusion compared to ocular hypertensives and normal volunteers.

3. To detect and quantify changes in ONH and peripapillary retinal blood flow by SLDF in open angle glaucoma and ocular hypertension following therapeutic intraocular pressure reduction.

   Hypothesis: OAG patients will show improvement in ocular perfusion compared to ocular hypertensives following similar and sustained therapeutic intraocular pressure reduction.

4. To examine the correlation between finger blood flow and changes in ONH blood flow following therapeutic IOP reduction in OAG and OHT.

   Hypothesis: OAG and OHT patients with peripheral vasospastic response will demonstrate different ONH blood flow changes in response to similar therapeutic IOP reduction when compared to non-vasospastic patients.

5. To assess ONH topographic changes following sustained therapeutic IOP reductions. These changes will be compared to those found in a group of ocular hypertensives undergoing similar IOP reduction.

   Hypothesis: OAG and OHT patients will show different ONH topographic changes following similar therapeutic IOP reductions.

6. To correlate changes in ONH topography and blood flow following therapeutic IOP reduction with central corneal thickness (CCT).

   Hypothesis: OAG and OHT patients with thinner corneas will show a different topographic and blood flow response to therapeutic IOP reduction compared to those with thicker corneas.

7. To determine prospectively whether changes in neuroretinal rim blood flow (objective #3), systemic vasospastic response (objective #4), CCT measurements (objective #5) and lamina cribrosa mechanical compliance (objective #6) in OAG and OHT were linked to long-term visual field progression.

   Hypothesis: Long term visual field progression in both OAG and OHT will be correlated with reduced neuroretinal rim blood flow, thinner corneas, the presence of vasospasticity and altered ONH biomechanics.

STUDY DESIGN

A. Patients

Patients will be recruited from the glaucoma clinics of Maisonneuve-Rosemont Hospital in Montréal (6782 patient visits in 2002-03), from the General/Emergency Ophthalmology Clinics of the same hospital (20,238 visits in 2002-03). Patients with OAG or OHT requiring initial or further IOP reduction will be recruited. Patients could be currently on topical medical therapy. One eye will be studied per patient. Normal subjects will be recruited from spouses and friends of recruited patients.

B. Inclusion Criteria and Patient Definitions

- Recruited patients with OAG should have gonioscopically open angles and fulfilled at least two of the following three criteria: history of intraocular pressure (IOP) above 21 mmHg, characteristic nerve fiber bundle visual field defects and an ONH showing glaucomatous disc changes. Age 40-80 years. Early glaucoma is arbitrarily defined as a mean defect of -2 to -6.9 db and moderate glaucoma as mean defect of -7.0 to -12.0 db. There is no restriction as to IOP at time of diagnosis.
- Recruited patients with OHT should have a history of IOP above 24 mmHg on at least two occasions, normal standard automated perimetry and an ONH appearance that ranged from normal to suspect but showed no localized thinning, saucerization, notching or evidence of progression.

- OAG and OHT patients were considered uncontrolled when their current IOP was higher than their target IOP and therefore were candidates for further therapeutic intervention.
- Only patients achieving a minimum of 20% reduction of IOP post-therapy as well as a minimum of four weeks follow up were eligible to complete the study.
- Age and sex-matched normal subjects will be recruited from spouses and friends of patients attending the ophthalmology clinic. They should have an IOP below 21 mmHg, normal visual field and normal appearance of ONH with no characteristics suspicious of glaucomatous optic neuropathy. They also should have normal ocular examinations and no family history of glaucoma.
- Subjects must have ocular media clear enough to permit imaging, and good fixation in the study eye in order to ensure good centration during flowmetry. Best corrected visual acuity must be at least 20/30 in the study eye.
- Measurements will be performed on one eye of each subject. The study eye will be chosen based on media clarity, larger neuroretinal rim area and better fixation with the fellow eye. When there was no difference between eyes in these criteria, one eye will be selected randomly.
- Subjects must be willing to participate in the study and give an informed consent to the recruiting physician.

C. Exclusion Criteria

- Non-glaucomatous ocular disease except pseudophakia, mild background diabetic retinopathy or mild macular disease not affecting fixation.
- Significant media opacities or poor tear film quality precluding SLDF or CSLO imaging.
- Refractive errors exceeding 6D equivalent sphere or 3D of astigmatism.
- Topography or flowmetry images of poor quality or unsuitable for analysis.

D. Study Procedures

Following informed consent, patients will undergo the procedures outlined below in a baseline visit and one or more follow up visits. IOP reduction will be achieved by medical, laser, or surgical intervention. All patients included in the study should have a sustained IOP reduction of 20% or more, as well as a minimum of four weeks follow up between the intervention and the follow up visit. For patients undergoing filtering procedures, the second imaging session will be at least 12 weeks following the intervention, in order to avoid issues of hypotony, edema and inflammation.

Normal subjects will undergo the baseline visits only with no visual field, finger Doppler, stereo photos or visual field testing.

1. Complete medical and ophthalmic history including family and reproductive history, and brachial blood pressure measurement and a complete ophthalmic exam noting the presence of a disc flame hemorrhage.

   Ocular perfusion pressure (OPP) is calculated from the formula:

$$OPP = 2/3\ (DBP + 1/3\ (SBP - DBP)) - IOP$$

where DBP = diastolic brachial blood pressure, SBP = systolic brachial blood pressure and IOP = intraocular pressure.

2. Stereo optic disc photos will be taken through dilated pupils with a stereo fundus camera at the baseline visit to assist in plotting the HRT contour line.

3. Assessment of ONH topography with confocal scanning laser ophthalmoscopy

   The Heidelberg Retina Tomograph (HRT, Heidelberg Engineering, GmbH, Heidelberg, Germany) version 2.01 will be used. Pupils less than 3mm diameter will be dilated. Technical aspects of the instrument were detailed in Chapter 1.

   Triplicate images will then be taken both at baseline and post-IOP reduction. The disc margin will be outlined manually with reference made to the stereo disc photos and the contour line will be automatically transferred to the post-intervention image. This approach allows assessment of individual intrasession variability and allows us to distinguish true change from intersession variability. ONH parameters will be calculated using the manufacturer's software. Change in ONH topographical parameters will be calculated by the software in real measurement units.

4. Assessment of ONH and peripapillary retinal blood flow with scanning laser Doppler flowmetry

The Heidelberg Retina Flowmeter (HRF, Heidelberg Engineering, GmbH, Heidelberg, Germany) with automatic full-field perfusion image analysis (AFFPIA) software [version 3.3] will be used. Technical aspects of the instrument were detailed in chapter 1. Pupils 3 mm in diameter or smaller will be dilated. A total of 7-9 evenly illuminated and centered ONH images will be acquired using a 2.5-degree x 10-degree frame and focusing on the superficial retina. The subject will be asked to use the fellow eye for fixation and to refrain from movement and blinking during image acquisition.

Prior to data analysis the best five images in terms of focusing, centration, brightness and absence of movements will be chosen. AFFPIA will then be performed on each of the five images with perfusion values for the neuroretinal rim, temporal peripapillary retina and nasal peripapillary retina given in arbitrary units (au)

5. Assessment of peripheral vasospasticity with finger laser Doppler flowmetry

Finger blood flow will be measured with the BLF 21 Laser Doppler Flowmeter (Transonic Systems Inc., Ithaca, NY). Compared to normals, vasospastic subjects show a lower blood flow at baseline, a larger increase in flow in response to warm water (while maintaining sub-normal values), and a much larger decrease in flow in response to cold water immersion. A response will be considered to be vasospastic when the ratio of maximum to minimum flow exceeds 7. Low flow (defined as

flows less than 50 tissue perfusion units (TPU) during the warm water phase is also indicative of vasospasm.

6. Automated Perimetry

Automated perimetry will be performed with the Humphrey Field Analyzer (Humphrey Instruments, San Leandro CA) with a white Goldman size III stimulus, using program 24-2 and SITA Standard strategy. Test time averages 6 minutes per eye. A pair of reliable fields will serve as a baseline and the Glaucoma Change Probability Analysis will be used to flag test points that are significantly deteriorated compared to baseline.

7. Pachymetry

Central corneal thickness (CCT) will be determined using five repeat measurements with an ultrasound corneal pachymeter (DGH 500 Pachette, DGH Technology, Fraser, PA, USA). The three closest values are averaged to give the CCT.

E. Clinical Management & Timing of Post-IOP Reduction Tests

Patients will be managed by current standards of care. Medical therapy, laser trabeculoplasty or trabeculectomy will be performed as clinically indicated. The minimal reduction of IOP to be eligible for post-IOP reduction imaging will be 20%. Patients will undergo the post-IOP reduction testing 4-6 weeks following the initiation of successful therapy.

F. Data Management

Baseline and follow-up data will be entered into an Excel spreadsheet. Statistical analyses will be performed using the appropriate tests.

G. Power and Sample Size Calculation

The estimate of sample size is based on an alpha of 0.05 and a power of 80% (beta of 0.20) using a two-sided sample t-test.

For the hypotheses related to ONH topographic changes and based on group standard deviations for change of mean cup depth following IOP reduction of 34 microns for the OHT group and 36 microns for the OAG group, a sample size of 20 patients per group is required to detect a difference of 32 microns.

For the hypotheses related to blood flow changes and based on group standard deviations for change of blood flow following IOP reduction of 86 au for the OHT group and 87 au for the OAG group, a similar sample size of 20 patients per group is required to detect a difference of 79 arbitrary units.

However, due to the fact that adequate blood flow data cannot be collected from all patients and assuming that around 20-30% of patients will be excluded at the baseline visit or during the study period, we will need to recruit around 25 OHTs and 25 OAGs to have adequate power to reveal meaningful correlations.

We will also recruit 25-30 age-matched normals for baseline blood flow comparisons with OAGs and OHTs.

H. Ethical Considerations

Approval will be obtained by the ethics committee of Maisonneuve-Rosemont Hospital. Prospective candidates will be thoroughly informed of the protocol and will have the opportunity to have any questions answered before being asked for informed consent. Normal volunteers will be informed of the minimal risks of short term topical dilating drops and of testing. Each patient will be given a code and all data will be masked to protect patient confidentiality.

Ocular exams, HRT, pachymetry and automated perimetry are part of standard care. Retinal, optic nerve and finger flowmetry are experimental procedures that have been approved as adhering to ANSI standards for human testing and pose minimal risk to subjects. The potential benefits are that these studies will contribute towards a better understanding of their disease and, eventually, to better management.

I. Study limitations

- In the presented studies, we will attempt to recruit all subjects having clear media and good fixation, as assessed at the slit lamp and by the patient's ability to adequately perform automated perimetry. However, in our pilot data good quality images were not attained in 1/4 to 1/3 of those initially considered as good candidates. This observation suggests that the current technique is not uniformly applicable in clinical practice.
- Study groups (OAG vs. OHT) might demonstrate significant differences in parameters such as age, IOP, ocular perfusion pressure, C/D ratio and visual field

mean defect. Such differences might also have an impact on group comparisons or might affect perfusion data and should be taken into consideration.

- Use of systemic vasoactive drugs (beta-blockers, ACE-inhibitors, Calcium channel blockers, etc) could also impact the results of ocular blood flow studies. Excluding patients on these medications would severely limit recruitment for the study and, more importantly limit general applicability of the results, since the majority of glaucoma patients are using vasoactive drugs. However, patients who change their vasoactive medications between baseline and post-IOP-reduction visits will be excluded.
- Study groups will include uncontrolled OAG and OHT patients, some of whom are newly diagnosed receiving no treatment and others are uncontrolled despite topical glaucoma therapy. Not all patients will have the same treatment, i.e. some will be treated by topical therapy and some will undergo laser trabeculoplasty in addition to topical therapy, while others will require filtering procedures. The influence of treatment type on topography measurements and blood flow measurements should be taken into consideration.

J. Funding and disclosure

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The authors have no proprietary interest in the Heidelberg Retina Tomograph, the Heidelberg Retinal Flowmeter, the AFFPIA software or the Transonic laser Doppler Flowmeter.

Chapter 3

Reproducibility of Retinal and Optic Nerve Head Perfusion Measurements Using Scanning Laser Doppler Flowmetry

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The authors have no proprietary interest in the Heidelberg Retina Flowmeter or the SLDF analysis software version 3.3.

ABSTRACT

Purpose: To evaluate the reproducibility of full-field perfusion analysis using scanning laser Doppler flowmetry (SLDF) for perfusion measurements of the neuroretinal rim of the optic nerve head and the peripapillary retina in open angle glaucoma and ocular hypertension patients as well as in normal subjects.

Methods: SLDF perfusion measurements of the neuroretinal rim and the peripapillary retina were performed on 20 patients with open angle glaucoma or ocular hypertension (Group G) and on 20 normal volunteers (Group N), using automatic full-field perfusion image analysis. Each subject underwent two independent sessions, thirty minutes apart, each involving five high quality images. Intra and intersession reproducibility coefficients for flow, volume and velocity were calculated for a single image and for means of three and five images using analysis of variance (ANOVA) models.

Results: Using a mean of five measurements for the parameter flow, the *intra*session coefficient of reliability in "Group G" was 0.99 for each of the rim, nasal retina and temporal retina. "Group N" showed intrasession coefficient of reliability of 0.93, 0.93 and 0.95 for the respective regions. The *inter*session coefficient of reliability for flow in "Group G" using a mean of five measurements was 0.99 for the rim, 0.95 for the nasal retina and 0.87 for the temporal retina. In "Group N", it was 0.87, 0.82, and 0.80 for the respective regions. In general, compared to single image analysis, intra and intersession reproducibility was better for the mean of three images and substantially better when the mean of five images was used.

Conclusion: SLDF full-field perfusion analysis is markedly more reproducible than the reported reproducibility of the original software using 10x10 pixels windows. Obtaining mean values for at least three images improves the intrasession and intersession reproducibility of this technique.

Key Words:

Glaucoma - Scanning Laser Doppler Flowmetry - Optic Nerve Blood Flow - Reproducibility.

INTRODUCTION

Substantial evidence suggests that defective perfusion of the optic nerve head (ONH) and peripapillary retina contributes to the development and progression of glaucomatous optic neuropathy in many patients.[1-4] Abnormalities of ocular blood flow in glaucoma have been shown using many techniques including fluorescein angiography,[5] color Doppler imaging,[6,7] laser Doppler flowmetry,[8] and pulsatile ocular blood flow.[9] The ability to accurately determine the perfusion of the ONH is fundamental to furthering our understanding of the pathogenesis of ONH damage in glaucoma.[10,11]

In 1992, Riva et al[12] introduced single-point laser Doppler flowmetry to obtain rapid and non-invasive hemodynamic measurements in the ONH. Since then, a number of studies have reported measurements of blood flow with this method in the ONH, [13,14] retina[15,16] and choroid.[17,18] The technique has been shown to have good reproducibility. It is thought to measure blood flow in the superficial layers of the disc and not in the deeper layers of the ONH.[19,20]

In 1995, Michelson and Schmauss[21] described the scanning laser Doppler flowmeter (SLDF), a non-invasive device that combines the laser Doppler flowmeter with a scanning laser system. It allows the visualization of perfused vessels in a scan area of 2.7 mm x 0.7 mm as well as the quantification of perfusion parameters in discrete locations of the ONH and peripapillary retina. In an experimental setup, SLDF was

shown to be both valid and reproducible. Michelson and associates[22] were able to estimate the capability of SLDF to measure the velocity of a moving plane in absolute units. Chauhan and Smith[23] conducted a series of experiments using fluids driven over a range of pump flow rates into glass capillaries and reported a linear relationship between SLDF measured flow and actual flow within a given operating range.

When first introduced, SLDF software permitted analysis by placement of a measurement window of variable size (1x1, 4x4, or 10x10 up to 50x50 pixels) in the region of interest within the scan area. Using this software, reproducibility of the neuroretinal rim blood flow was considered poor with an intersession coefficient of reliability of 0.36 in glaucoma patients and 0.47 in normal volunteers,[24] although peripapillary retinal blood flow appeared to be more reproducible.[22,24,25] Investigators attributed the large variation in rim blood flow to focusing difficulties on the neuroretinal rim due to its low reflectivity.[24] Such an effect can reduce the power to detect statistical differences between various groups of patients or following experimental manipulation of ONH blood flow.

In 1998, Michelson and coworkers[26] described a new method for automatic full-field perfusion image analysis (AFFPIA). They developed an algorithm to improve SLDF measurements in the ONH and peripapillary retina by reducing the influence of heterogeneity of the perfusion map as well as of heart beat-associated pulsations. As opposed to conventional analyses of SLDF images by a measurement window of 10x10

pixels whose position is selected by the operator, this technique calculates the perfusion parameters of all valid pixels in the entire scan area and then computations are performed using the perfusion values retained for analysis. The resulting perfusion map is subdivided into temporal and nasal peripapillary retinal areas as well as neuroretinal rim area.

Michelson and coworkers[26] reported intersession coefficients of reliability for the flow parameter in the nasal and temporal peripapillary retina of 0.76 and 0.73 respectively in normal subjects. This intersession reliability was in the same range as the reliability of the conventional analysis using measurement windows.

In a recent study, Iester et al[27], using AFFPIA, reported an increased variability in the measurements of the neuroretinal rim compared to the peripapillary retina. The authors reported an intra-image (same image analyzed 5 times by the same observer) coefficient of variation of 20.3% (range, 0.5-28%) and an inter-image (3 consecutive images analyzed once by the same observer) coefficient of variation of 24.4% (range, 2.0-30%). However, no reproducibility data has been reported using this technique when perfusion values were obtained from the mean of the multiple perfusion images.

The purpose of this study was to evaluate the intrasession and intersession reproducibility using the SLDF full-field perfusion analysis of the neuroretinal rim and peripapillary retina in our population of open angle glaucoma and ocular hypertension patients as well as in normal volunteers. We also examined the reproducibility when data was derived from multiple successive perfusion images in an attempt to determine

the optimal number of measurements needed to obtain highly reproducible perfusion values.

PATIENTS AND METHODS

The study was approved by the research committee of Maisonneuve-Rosemont Hospital, University of Montreal. An informed consent was obtained from each subject prior to enrollment.

Thirty-two patients with open angle glaucoma (OAG) or ocular hypertension (OHT) were recruited from the glaucoma clinic of the hospital. These were included under one group named "Group G". Twenty-nine normal subjects were also recruited, mostly from friends and spouses of glaucoma patients and volunteers, and were included in a second group named "Group N".

Patients with OAG had gonioscopically open angles and fulfilled two of the following three criteria: a history of intraocular pressure (IOP) greater than 21 mmHg, characteristic nerve fiber bundle visual field defects and glaucomatous optic neuropathy. Ocular hypertensives had a history of repeated IOP measurements greater than 24 mmHg with normal visual fields and normal ONH appearance. Normal subjects had IOP below 21 mmHg, with normal visual fields, normal ONH, and no family history of glaucoma.

Subjects were excluded from the study if they had other abnormal ocular findings, apart from pseudophakia, if significant media opacities precluding SLDF imaging were present, or if at any time they were unable to cooperate.

During the prestudy visit of each patient, medical and ocular history was taken. Best-corrected visual acuities, intraocular pressures and refractive errors were measured. A routine ophthalmologic examination including biomicroscopy, gonioscopy and ophthalmoscopy was performed and a recent automated perimetry (Humphrey Field Analyzer, program 24-2, Humphrey Instruments, San Leandro, California) was used to evaluate the visual field. Blood pressure and heart rate were recorded. Ocular perfusion pressure (OPP) was calculated according to the formula:

OPP = 2/3 [Diastolic Blood Pressure + 1/3 (Systolic Blood Pressure - Diastolic Blood Pressure)] – IOP.[28]

The SLDF used in this study (Heidelberg Retina Flowmeter, HRF, Heidelberg Engineering GmbH, Heidelberg, Germany) is a non-invasive instrument combining both a laser Doppler flowmeter with a scanning laser technique. It measures the amount of backscattered light at different locations in the tissue of interest within a short period of time. An infrared diode laser with wavelength of 780 nm is used. The area examined measures 2.7 mm x 0.7 mm in size, composed of 64 horizontal lines, each with 256 points giving an approximate spatial resolution of 10 µm. Each line is scanned sequentially a total of 128 times with a total acquisition time of 2.05 seconds. A discrete fast Fourier transformation is performed over the 128 intensity values for each retinal point, generating a spectrum of the Doppler shift for each point. A two-dimensional map of microvascular perfusion of the area to be studied is thus obtained.

The automatic full-field perfusion image analysis software (SLDF-version 3.3) (Figure 3.1) developed by Michelson and associates[27] enhances the computations previously generated by the HRF through:

- Elimination of all pixels with incorrect brightness (overexposed, underexposed) and pixels of cup area as well as marking of eye movements (saccades) that lead to erroneous perfusion data.
- Exclusion of retinal vessels with a diameter greater than 30 μm, whose flow velocities are too high for the sampling frequency afforded by this technique.
- The analysis is based on the average of all valid pixels in the scan area rather than data obtained from a discrete target square.
- The perfusion map can be subdivided into 3 critical regions of interest for analysis, namely the temporal and nasal peripapillary retinal areas as well as the rim area, and each can be analyzed separately.
- Heart beat associated pulsation of capillary blood flow is estimated by plotting the mean flow of each horizontal line against time.
- All data and settings (position of the rim circles, calculation parameters) used in an analysis are saved and can be retrieved for subsequent follow-ups or reanalysis.

Full-field perfusion analysis of HRF images is thus obtained, from which numerical readings of "flow" (distance traveled by all moving red blood cells per unit of time), "volume" (number of moving red blood cells) and "velocity" (mean red blood cell speed) are given in arbitrary units (AU).

For testing, pupils 3 mm in diameter or smaller were dilated. The subject was asked to use the fellow eye for fixation on a target and to refrain from movement and blinking during image acquisition. The fundus camera was adjusted until a well focused, evenly illuminated and centered view of the ONH was obtained. A total of 7-9 images were then acquired sequentially at each of two independent sessions 30 minutes apart, focussing on the superficial retina. In order to obtain a consistent and optimum exposure within or between sessions, illumination intensity was adjusted to the maximum level that did not result in overexposed pixels on the peripapillary retina. Also, the angulation of the fundus camera as well as its distance from the eye was kept constant throughout the process of image acquisition.

All images were reviewed by the same observer, and prior to data analysis, the best five images from each session in terms of focussing, centration, brightness and absence of movements were chosen. Subjects with poor quality images were excluded from the study. Each of the five chosen images was then analyzed for measurements of blood flow, volume and velocity for the nasal and temporal peripapillary retinal areas, as well as the neuroretinal rim area.

Statistical evaluations were then performed on both groups for flow, volume and velocity using analysis of variance (ANOVA). The reproducibility of the measurements was assessed by estimating intraclass correlation coefficients according to two random effects ANOVA models. The intraclass correlation coefficient is considered to be the

standard statistical indicator of reproducibility based on the classical test theory developed by Spearman.[29] It is defined as $\sigma_T^2/(\sigma_T^2 + \sigma_e^2)$, where σ_T^2 is the component of "true" variance (variance between subjects) while σ_e^2 is the component of variance reflecting measurement error (variance within subjects or "noise"). If the noise is small relative to the true variation, the value of the intraclass correlation coefficient will be closer to 1.0, thus reflecting a high level of reproducibility. Similarly, an intraclass correlation coefficient of 0.5 indicates that only half of all variability is due to real differences between subjects while the other half is due to noise.

Intrasession reproducibility: The data from each session was analyzed by adjusting one-way random effect ANOVA models and calculating three intraclass correlation coefficients. R1 is the intraclass correlation coefficient for a single measurement (image). R3 and R5 are obtained by applying the Spearman-Brown formula (introduced by Lord and Novik). [30] They correspond to stepped-up intraclass correlation coefficients for the mean of 3 and 5 measurements (images) respectively. In addition, within-subject variation was studied by calculating for each subject the coefficient of variation for the mean of 5 measurements from the first session. Mean (± SD) coefficients of variation were then calculated for both groups.

Intersession reproducibility: Mixed two-way ANOVA models were adjusted to estimate intraclass correlation coefficients R1, R3 and R5. For R1 and R3, images were selected at random from the set of 5 images available for each session. Changes between sessions for the mean of 5 measurements were also examined by computing for

each subject the percent change in mean values relative to the mean value from the first session. Mean (± SD) percent changes were then calculated for both groups. Since only two sessions were performed, mean percent changes rather than coefficients of variation were calculated to estimate the intersession variability.

The precision of the estimated coefficients of reliability was then ascertained by computing 95% confidence intervals (CI). Confidence intervals were used to assess whether differences between coefficients of reliability for a single image, a mean of 3 or a mean of 5 were statistically significant by examining whether or not their confidence intervals overlap. Confidence intervals were also used to assess a statistically significant difference in coefficients of reliability between the two study groups.

RESULTS

SLDF measurements were performed on 32 patients with OAG or OHT and 29 normal subjects. Good quality images were obtained from 24 OAG/OHT patients and 21 normal subjects and were analyzed. From these, four OAG patients and one normal subject were excluded for invalid rim data. Thus data is presented for 20 OAG/OHT patients (Group G) and 20 normal volunteers (Group N).

Causes of poor quality images were primarily excessive eye movements and media opacities in the cornea, lens or vitreous. Invalid rim data was the term generated by the software in cases where the extremely low reflectivity of the ONH usually accompanied by high cup/disc (C/D) ratio caused inadequate number of pixels in the neuroretinal rim tissue to be available for analysis. From the four OAG patients excluded for invalid rim data; three had C/D ratio >0.8. The glaucoma patient with C/D ratio <0.8 and the normal subject (with C/D ratio of 0.3) had an unusually low reflectivity of the neuroretinal rim. All five subjects were automatically excluded by the SLDF software.

The mean (± standard deviation, SD) age was 65.9 ± 12.4 years for "Group G" (age range 42-80 years) and 63.6 ± 9.8 years for "Group N" (age range 41-77 years). No statistically significant difference in age was detected between both groups, $P = 0.46$, [two-tailed Student t-test]. There were 10 males (50%) and 10 females (50%) in "Group G", and 9 males (45%) and 11 females (55%) in "Group N". "Group G" included 16 patients (80%) with OAG and 4 patients (20%) with OHT.

Six subjects (30%) in "Group G" and four subjects (20%) in "Group N" had medically controlled systemic hypertension. Three subjects (15%) in "Group G" and two subjects (10%) in "Group N" had diabetes mellitus.

OAG and OHT patients within Group G were heterogeneous as regards the control of their disease. Nineteen of the 20 subjects in this group were receiving topical hypotensive therapy. Of the 20 subjects in Group G, as evaluated by their IOP, ONH appearance and visual field, 17 subjects (85%) were considered uncontrolled while only 3 subjects (15%) had adequately controlled IOP. The average (± SD) mean defect on automated perimetry was -7.05 (± 7.84) in this group with a range of +0.26 to -27.9. One patient in "Group G" (5%) and three subjects (15%) in "Group N" had previous cataract surgery in the study eye, one patient in "Group G" (5%) had previous glaucoma surgery and one patient (5%) had a previous combined cataract and glaucoma surgery. The means (± SD) of age, IOP, clinical C/D ratio and OPP of the subjects in both study groups are shown in Table 3.1. Statistically significant differences between "Group G" and "Group N" were demonstrated in two of the four parameters ($P \leq 0.001$, two-tailed Student t-test). Marginally insignificant differences were reported between the two study groups in the means of OPP ($P = 0.08$).

INTRASESSION REPRODUCIBILITY

In "Group G", the coefficient of reliability of a single image for the parameter flow for each region (rim, nasal retina and temporal retina) was 0.94. Coefficients of reliability

for flow using a mean of three and five images were 0.98 and 0.99 respectively for each region (Table 3.2).

In "Group N", the coefficient of reliability of a single image for the parameter flow in the rim was 0.54, whereas for the nasal and temporal peripapillary retina it was 0.67 and 0.80 respectively. However, when using a mean of three and five images the coefficient of reliability for flow improved to a range of 0.78-0.92 and 0.93-0.95 respectively, depending on location (Table 3.3).

Overall, the intrasession coefficient of reliability of 5 images for the three areas tested was higher in "Group G" than in "Group N" (for the parameter flow 0.99 versus 0.93-0.95, for volume 0.97-0.99 versus 0.94-0.97 and for velocity 0.98-0.99 versus 0.88-0.96 respectively). In many locations this difference was statistically significant as indicated by the non-overlap of the 95% confidence intervals.

Intrasession variability of the parameter flow as measured by mean coefficients of variation for five perfusion images revealed 9.5%-19.8% variability in "Group G" as compared to 11.4%-16.4% variability in "Group N" depending on location (Table 3.4). Higher intrasession mean coefficient of variation for the rim than for the peripapillary retina was present in all parameters in both study groups. The variability was not uniform in either group with some subjects demonstrating small variations in measurements and others showing large variations (data not shown).

INTERSESSION REPRODUCIBILITY

In "Group G", the intersession coefficient of reliability of a single image for the parameter flow was 0.99 for rim, 0.95 for nasal retina and 0.73 for temporal retina (Table 3.5). Coefficient of reliability for virtually all parameters was higher, though not significantly so, when a mean of three or five images was used.

In "Group N", the coefficient of reliability of a single image for the parameter flow was 0.45 for the rim, while for the nasal and temporal peripapillary retina it was 0.75 and 0.42 respectively (Table 3.6). However, when using a mean of three or five measurements, the coefficient of reliability improved to a range of 0.84-0.87 and 0.80-0.87 respectively, depending on location. Volume and velocity parameters showed similar patterns of improved reliability when multiple images were used for analysis (Table 6).

Intersession variability of the parameter flow, as measured by mean percent change for five perfusion images, revealed 9.5%-10.6% variability in "Group G" as compared to 9.8%-12.5% variability in "Group N" depending on location (Table 3.7).

In both study groups, intersession mean percent change for the rim was generally comparable to that of the peripapillary retina. Again, the variability was not uniform among subjects in either group, with some subjects demonstrating small variations in measurements and others showing large variations (data not shown).

Table 3.1. Intraocular Pressure, Cup/Disc Ratio and Ocular Perfusion Pressure in "Group G" and "Group N".

Parameter	GROUP G (N=20)		GROUP N (N=20)		P value
	Mean	SD	Mean	SD	
Age	65.9	12.4	63.6	9.8	0.460
I.O.P.	22.6	5.63	16.9	2.65	0.001
C/D ratio	0.6	0.23	0.2	0.12	0.000
O.P.P.	44.29	6.60	48.19	7.18	0.063

Group G = open angle glaucoma and ocular hypertension patients, Group N = normal subjects, I.O.P. = intraocular pressure, C/D ratio = cup/disc ratio, O.P.P. = ocular perfusion pressure. Two-tailed Student's t-test.

Table 3.2. Intrasession Coefficients of Reliability (R) and 95% Confidence Intervals (C.I.) for "Group G" (N=20) as calculated for one image (R1), mean of three images (R3) and mean of five images (R5).

Parameter	Location	Perfusion Values		Coefficient of Reliability		
		Mean	S.D.	R1 (95% C.I.)	R3 (95% C.I.)	R5 (95% C.I.)
Volume	Nasal	20.62	7.26	0.93 (0.85-0.97)	0.97 (0.95-0.99)	0.99 (0.98-0.99)
	Rim	17.43	7.21	0.86 (0.74-0.94)	0.95 (0.89-0.98)	0.97 (0.95-0.99)
	Temporal	21.75	9.49	0.93 (0.85-0.97)	0.97 (0.95-0.99)	0.98 (0.97-0.99)
Flow	Nasal	351.0	165.5	0.94 (0.88-0.97)	0.98 (0.96-0.99)	0.99 (0.98-1.00)
	Rim	256.6	195.6	0.94 (0.88-0.97)	0.98 (0.95-0.99)	0.99 (0.98-1.00)
	Temporal	337.5	129.0	0.94 (0.87-0.97)	0.98 (0.95-0.99)	0.99 (0.97-0.99)
Velocity	Nasal	1.27	0.53	0.95 (0.89-0.98)	0.98 (0.96-0.99)	0.99 (0.98-1.00)
	Rim	1.20	0.71	0.92 (0.84-0.96)	0.97 (0.94-0.99)	0.98 (0.96-0.99)
	Temporal	1.20	0.42	0.95 (0.91-0.98)	0.98 (0.97-0.99)	0.99 (0.97-0.99)

Table 3.3. Intrasession Coefficients of Reliability (R) and 95% Confidence Intervals (C.I.) for "Group N" (N=20) as calculated for one image (R1), mean of three images (R3) and mean of five images (R5).

Parameter	Location	Perfusion Values		Coefficient of Reliability		
		Mean	S.D.	R1 (95% C.I.)	R3 (95% C.I.)	R5 (95% C.I.)
Volume	Nasal	18.09	3.98	0.76 (0.57-0.89)	0.90 (0.80-0.96)	0.94 (0.89-0.96)
	Rim	17.99	4.33	0.84 (0.69-0.92)	0.94 (0.87-0.97)	0.95 (0.91-0.99)
	Temporal	21.44	5.91	0.85 (0.72-0.93)	0.95 (0.89-0.98)	0.97 (0.94-0.99)
Flow	Nasal	279.1	79.9	0.67 (0.44-0.84)	0.86 (0.70-0.94)	0.93 (0.86-0.97)
	Rim	272.0	92.9	0.54 (0.28-0.76)	0.78 (0.54-0.91)	0.93 (0.87-0.97)
	Temporal	307.5	85.5	0.80 (0.63-0.91)	0.92 (0.84-0.97)	0.95 (0.91-0.98)
Velocity	Nasal	1.04	0.27	0.69 (0.47-0.85)	0.87 (0.72-0.94)	0.93 (0.87-0.97)
	Rim	1.17	0.28	0.61 (0.36-0.80)	0.82 (0.63-0.92)	0.88 (0.78-0.95)
	Temporal	1.10	0.29	0.76 (0.58-0.89)	0.91 (0.80-0.96)	0.96 (0.92-0.98)

Table 3.4. Intrasession variability of five images as measured by mean (± SD) coefficients of variation according to parameter, location of measurement and study group.

Parameter	Location	Mean (± SD) Coefficients of Variation	
		GROUP G (N = 20)	GROUP N (N = 20)
Volume	Nasal	7.7 ± 4.1	8.8 ± 6.0
	Rim	14.4 ± 8.7	10.0 ± 5.5
	Temporal	7.8 ± 3.6	9.4 ± 4.6
Flow	Nasal	9.5 ± 5.3	11.4 ± 7.7
	Rim	19.8 ± 15.9	16.4 ± 12.3
	Temporal	10.3 ± 5.1	11.6 ± 5.4
Velocity	Nasal	9.6 ± 5.7	11.2 ± 7.0
	Rim	22.9 ± 16.8	13.8 ± 10.1
	Temporal	9.0 ± 4.0	11.5 ± 4.6

Group G = open angle glaucoma and ocular hypertension patients, Group N = normal subjects

Table 3.5. Intersession Coefficients of Reliability (R) and 95% Confidence Intervals (C.I.) for "Group G" (N=20) as calculated for one image (R1), mean of three images (R3) and mean of five images (R5).

Parameter	Location	Coefficient of Reliability		
		R1 (95% C.I.)	R3 (95% C.I.)	R5 (95% C.I.)
Volume	Nasal	0.91 (0.80-0.97)	0.93 (0.84-0.97)	0.95 (0.88-0.98)
	Rim	0.90 (0.76-0.96)	0.92 (0.81-0.97)	0.95 (0.88-0.98)
	Temporal	0.84 (0.63-0.95)	0.92 (0.80-0.97)	0.93 (0.83-0.97)
Flow	Nasal	0.95 (0.88-0.98)	0.96 (0.89-0.98)	0.95 (0.89-0.98)
	Rim	0.99 (0.98-1.00)	0.98 (0.95-0.99)	0.99 (0.97-1.00)
	Temporal	0.73 (0.44-0.89)	0.86 (0.68-0.94)	0.87 (0.71-0.95)
Velocity	Nasal	0.94 (0.86-0.98)	0.95 (0.88-0.98)	0.96 (0.89-0.98)
	Rim	0.85 (0.66-0.94)	0.95 (0.87-0.98)	0.96 (0.90-0.98)
	Temporal	0.78 (0.53-0.91)	0.89 (0.75-0.96)	0.89 (0.75-0.96)

Table 3.6. Intersession Coefficients of Reliability (R) and 95% Confidence Intervals (C.I.) for "Group N" (N=20) as calculated for one image (R1), mean of 3 images (R3) and mean of 5 images (R5).

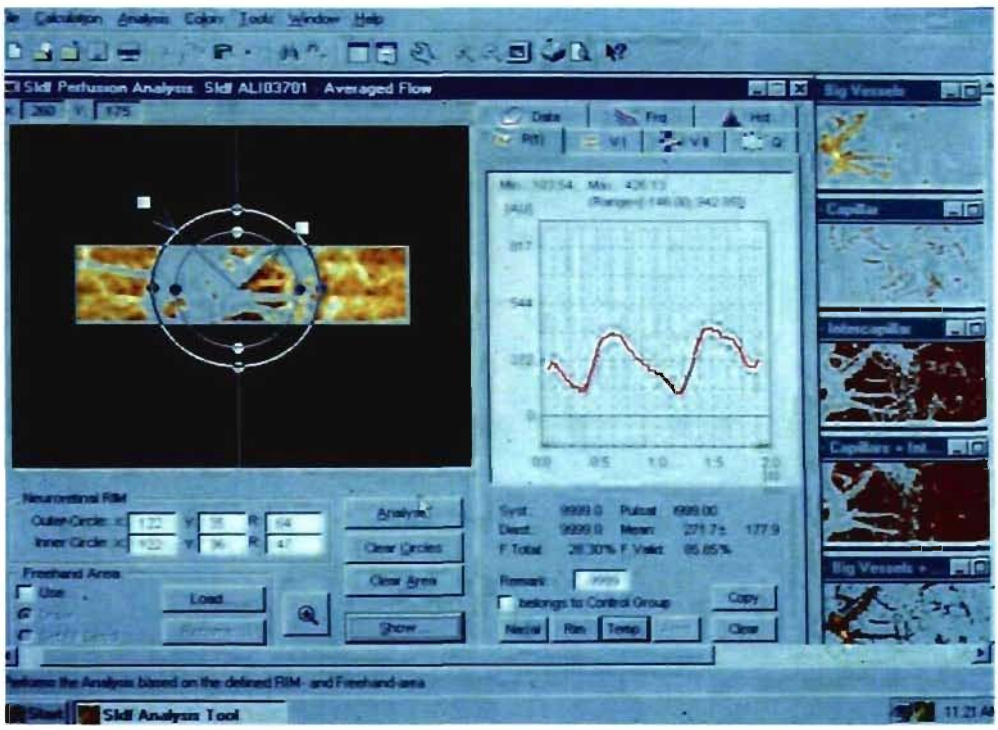
Parameter	Location	Coefficient of Reliability		
		R1 (95% C.I.)	R3 (95% C.I.)	R5 (95% C.I.)
Volume	Nasal	0.85 (0.67-0.94)	0.88 (0.71-0.95)	0.85 (0.65-0.94)
	Rim	0.54 (0.14-0.79)	0.86 (0.68-0.94)	0.82 (0.59-0.92)
	Temporal	0.80 (0.57-0.92)	0.93 (0.83-0.97)	0.92 (0.81-0.97)
Flow	Nasal	0.75 (0.47-0.89)	0.87 (0.69-0.94)	0.82 (0.59-0.92)
	Rim	0.45 (0.03-0.74)	0.87 (0.69-0.94)	0.87 (0.68-0.95)
	Temporal	0.42 (0.00-0.72)	0.84 (0.64-0.93)	0.80 (0.56-0.92)
Velocity	Nasal	0.78 (0.53-0.91)	0.88 (0.73-0.95)	0.83 (0.63-0.93)
	Rim	0.28 (0.00-0.64)	0.79 (0.54-0.91)	0.67 (0.33-0.85)
	Temporal	0.59 (0.21-0.82)	0.84 (0.65-0.93)	0.82 (0.61-0.93)

Table 3.7. Intersession variability of the mean (± SD) of five images as measured by mean percent change according to parameter, location of measurement and study group.

Parameter	Location	Mean (± SD) Percent Change	
		GROUP G (N = 20)	GROUP N (N = 20)
Volume	Nasal	10.4 ± 6.4	9.1 ± 8.6
	Rim	10.7 ± 8.6	11.0 ± 9.6
	Temporal	7.4 ± 7.2	7.0 ± 7.6
Flow	Nasal	10.6 ± 6.7	10.3 ± 13.3
	Rim	9.7 ± 8.2	12.5 ± 12.0
	Temporal	9.5 ± 9.4	9.8 ± 14.2
Velocity	Nasal	9.7 ± 6.3	9.7 ± 11.5
	Rim	14.4 ± 9.7	15.4 ± 17.7
	Temporal	9.1 ± 8.8	8.8 ± 12.2

Group G = open angle glaucoma and ocular hypertension patients, Group N = normal subjects.

Figure 3.1.



SLDF full-field perfusion analysis showing: Top left: Flow image of temporal peripapillary retinal area after automatic exclusion of vessels with diameter more than 30 microns and outlining of the optic nerve head and the neuroretinal rim area (circles). Top right: Graphic representation of the heart beat-associated pulsation of capillary blood flow during the two-second scan. Bottom: Data relating to image processing and perfusion values.

DISCUSSION

SLDF represents a significant advance in the noninvasive evaluation of the microvascular hemodynamics of the ONH and peripapillary retina. However, it is important to determine its reproducibility, validity and limitations before its application to a clinical setting.

Our study was conducted on 61 subjects recruited from the glaucoma clinics of the hospital. However, we were unable to obtain good quality images on 16 of the 61 subjects while 5 subjects were excluded for invalid rim data as determined by the software. We attempted to recruit all subjects having clear media and good fixation, usually assessed by ability to adequately perform automated perimetry. However, we were unable to produce good quality images in one third of those initially considered as good candidates. This observation points to the applicability of the technique in clinical practice. However, for those subjects in whom the technique can be successfully applied, reproducibility was, in general, very high.

Using the 10x10 pixel measurement box placed in a retinal location of normal subjects, authors have reported intrasession reliability for the parameter flow of 0.84,[21] and intersession reliability ranging from 0.62 to 0.82.[22,24] However, with placement of the box in a rim location, the intersession coefficient of reliability for flow dropped to 0.47 in normal volunteers and 0.36 in glaucoma patients.[24] Using the same technique, reported intrasession coefficients of variation for flow in a retinal location of normal

subjects ranged between 6.6%[31] and 12%[32] while intersession coefficients of variation ranged between 14%[25] and 22%.[24] In a rim location, the reported intersession coefficient of variation was 25%.[24] In one study, using flow histograms and pixel by pixel analysis of the entire perfusion image, the intersession coefficient of variation for the parameter flow decreased from 30.1% to 16.3%.[26]

The technique of SLDF with the conventional evaluation of 10x10 pixel windows using the original HRF software has been reported to show the following limitations[19]:

- Artifacts caused by eye movements occurring during the 2 seconds of image acquisition disturb the Doppler shift signal.
- Subjective variation in the position of the measurement window selected by the operator between images.
- Placement of the 10x10 pixels measurement box on the neuroretinal rim of glaucoma patients with high cup/disc ratio and thin rim unavoidably includes an area of the cup or the peripapillary retina in the box thus leading to erroneous data.
- Clinically insignificant media opacities degrade the quality of the images as well as increase the underlying noise and thus artifactually increase the overall perfusion values.
- Monocular patients and patients with poor vision in the contralateral eye interfering with fixation cannot be tested.

The SLDF analysis software used in this study (originally developed by Michelson and associates) is believed to enhance the computations previously generated by the HRF

through elimination of the first three limitations. Using this full-field perfusion analysis, Michelson and associates reported intersession coefficient of reliability of 0.74 for flow in the retina in normal subjects.[27] This intersession reliability was in the same range as the reliability of the conventional analysis of the 10x10 pixels measurement window used in the original HRF software. However, Michelson and associates[27] used only one image and consequently one measurement per session for their computations.

The present study reports the first reproducibility data for SLDF full-field perfusion analysis when values were obtained from the means of multiple perfusion images and demonstrates that, using this method of analysis, reproducibility values are much higher than the reported values using the 10x10 pixels measurement window.

It is well established in the statistical literature that increasing the number of measurements is a good approach to improve reliability since the mean of several measurements is always more reliable than a single measurement.[29] Increasing the number of images (measurements) used in the analysis from one to five increased both intrasession (Tables 3.2 and 3.3) and intersession reliability (Tables 3.5 and 3.6), and did so significantly for intrasession reliability. With three images (measurements) improvements were still achieved, although not to a level that was statistically significant.

In comparison to previous literature, the present study also demonstrates improved intersession variability, whereas intrasession variability was similar to the previously reported range. It is believed that both the intrasession and intersession variation contain elements due to technical variables or measurement errors as well as elements due to true physiologic changes. In this study, all subjects were asked to sit back between the two sessions for an interval of 30 minutes in an attempt to maintain the same physiological status throughout the imaging process, thus allowing us to examine primarily the variation due to technical variables. These variables might account for the individual differences in reproducibility that were observed in the different subjects.

Reproducibility was generally better in "Group G" than in "Group N". However, this difference only reached statistical significance (as ascertained by the non-overlap of 95% confidence intervals), in the intrasession reliability for the parameters flow and velocity in the neuroretinal rim and nasal peripapillary retina (Tables 3.2, 3.3) and in the intersession reliability for the parameter flow in the neuroretinal rim (Tables 3.5, 3.6). Better reproducibility in glaucoma patients compared to normals was similarly reported by Nicolela et al.[24] It might be explained in part by the fact that glaucoma patients might have been better fixators as result of previous training (i.e. through repeated automated perimetry, optic disc photography and multiple slit-lamp examinations). However, we could not detect a difference between either study group in terms of image quality.

A more likely explanation lies in the way in which the intraclass correlation coefficient, R, assesses reliability. As discussed in Methods, $R = \sigma_T^2/(\sigma_T^2 + \sigma_e^2)$, where σ_T^2 is the amount of variance between subjects (true variance) and σ_e^2 is the amount of variance within subjects (noise). In Group N σ_T^2 is relatively small because all subjects have normal blood flow. On the other hand Group G, whose optic discs range from normal to advanced cupping, has a wider range of blood flow giving a larger σ_T^2. Due to differences in σ_T^2 between the two groups, σ_e^2 could be the *same* in both groups and yet yield a *larger* intraclass correlation coefficient for Group G. The perfusion values shown in Figures 2 and 3 indeed demonstrate a larger between-subject variation in Group G when compared to Group N, which explains the higher reliability estimates in that group.

Several authors have investigated the validity of SLDF for quantitative evaluation of retinal and ONH perfusion. Michelson and Schmauss reported a significant linear relationship between SLDF flow and ocular perfusion pressure while varying the IOP by a suction cup in normal volunteers.[21] Furthermore, they compared measurements of corresponding retinal points by SLDF and a commercially available single-point laser Doppler flowmeter and reported a significant and linear relationship for flow, volume and velocity in normal and glaucomatous eyes.[22] Other investigators[33,34] have shown that SLDF is appropriate for description of the effect of graded changes in blood gases on retinal hemodynamics. They noted that changes in measured blood flow at the ONH occurred in the expected direction in response to blood gas perturbations.

To conclude, SLDF analysis using HRF images permits non-invasive, high resolution mapping of perfused vessels and capillaries of the ONH and peripapillary retina. The original software, however, enables the quantification of blood flow, volume and velocity only in selected areas of the perfusion map with poor reproducibility in the neuroretinal rim.[24] Full-field perfusion analysis is a newer approach which significantly reduces variables caused by the patient, operator or device and which permits highly reproducible quantification and documentation of the entire perfusion map including the neuroretinal rim in glaucoma patients. Obtaining mean values from at least three images improves both the intrasession and intersession reproducibility of this technique.

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Chapter 4

Evaluation of Optic Nerve Head and Peripapillary Retinal Blood Flow in Glaucoma Patients, Ocular Hypertensives and Normal Subjects

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ABSTRACT

Purpose: To compare optic nerve head (ONH) and peripapillary retinal blood flow in subjects with open angle glaucoma (OAG), ocular hypertension (OHT), and normal eyes (NOR) using full-field perfusion analysis of scanning laser Doppler flowmetry (SLDF) images.

Design: Prospective, nonrandomized clinical trial.

Methods: Twenty uncontrolled OAG patients, twenty uncontrolled OHT patients and twenty normal volunteers were prospectively enrolled. Mean ONH and peripapillary retinal blood flow measurements were performed by SLDF version 3.3 using five Heidelberg Retina Flowmeter (Heidelberg Engineering, Heidelberg, Germany) images. Statistical evaluations were performed on the three study groups using one-way analysis of variance (ANOVA). Flow values of the neuroretinal rim of the ONH, nasal peripapillary retina and temporal peripapillary retina were then correlated with the clinical parameters of age, cup/disc (C/D) ratio, IOP, visual field mean defect, maximum-recorded IOP and ocular perfusion pressure.

Results: Neuroretinal rim blood flow in the OAG group was 158 ± 79 au whereas in the OHT group it was 277 ± 158 au and in the NOR group it was 272 ± 93 au. Differences were statistically significant between OAG group and each of the other groups [P = 0.001], but not between OHT and NOR groups [P = 0.91]. Peripapillary retinal flow values showed no statistically significant differences between groups [P = 0.76 nasal and 0.93 temporal]. Neuroretinal rim flow values showed a significant inverse correlation with C/D ratio [P = 0.001]. Mean neuroretinal rim blood flow was

significantly higher (350 ± 184 au) in the 10 OHT patients with C/D ratios < 0.4 when compared to the 10 OHT patients with larger C/D ratios (203 ± 79 au)[P = 0.039]. On the other hand, peripapillary retinal blood flow showed no significant correlation with any clinical parameter.

Conclusion: OAG patients had significantly lower blood flow in the ONH when compared to OHT patients and normal volunteers. No significant differences in ONH blood flow were found between ocular hypertensives and normal volunteers. For peripapillary retinal blood flow, no significant difference was seen between any groups. Neuroretinal rim blood flow was significantly inversely correlated to increased C/D ratio. Ocular hypertensives with larger C/D ratios demonstrated significantly lower rim blood flow compared to those with smaller C/D ratios, suggesting that rim perfusion might be reduced in high-risk ocular hypertensives prior to the manifestation of visual field defects.

INTRODUCTION

It is well established that elevated intraocular pressure (IOP) is an important risk factor in glaucoma. However, since elevated IOP alone is neither sufficient (in ocular hypertension, OHT) nor necessary (in normotensive glaucoma, NTG) for the development of glaucoma or its progression,[1-3] other causes of glaucoma have been investigated. Substantial evidence points to defective perfusion of the optic nerve head (ONH) as a risk factor to the development and progression of glaucomatous optic disc changes.[4,5]

Some of the main evidence implicating blood flow deficits in glaucoma is derived from fluorescein angiography. These studies[6-8] show delayed retinal circulation as well as impaired perfusion of the ONH, peripapillary retina and choroid of glaucoma patients. The severity of perfusion defects progresses with the severity of glaucoma and the defects correlate well with visual field loss and nerve fibre layer dropouts. Techniques using color Doppler imaging[9-13] and pulsatile ocular blood flow[14-17] demonstrated that both retrobulbar blood flow and bulk choroidal blood flow were reduced in glaucoma patients in comparison to normal subjects matched for age and circulatory risk factors.

Recently, scanning laser Doppler flowmetry (SLDF) has been reported to measure blood flow directly in the ONH, in a rapid and noninvasive fashion. The technique is based on the Doppler effect, in which moving red blood cells cause a shift in the frequency of the reflected laser beam.[18]

In 1998, Michelson and coworkers[19] described a new method for SLDF automatic full-field perfusion image analysis and reported intersession coefficients of reliability for the flow parameter in the nasal and temporal peripapillary retina of 0.76 and 0.73 respectively in normal subjects.

The aim of the present study is to evaluate blood flow in the neuroretinal rim of the optic nerve head and in the peripapillary retina using SLDF automatic full-field perfusion image analysis. Perfusion measurements using this technique are compared in open-angle glaucoma patients, ocular hypertensives, and normal subjects and then correlated with several clinical parameters.

PATIENTS AND METHODS

Twenty uncontrolled OAG patients (OAG Group) and twenty uncontrolled OHT patients (OHT Group) were recruited from the glaucoma clinics of the hospital into this cross-sectional study. Twenty normal subjects (NOR Group) were also recruited. An informed consent was obtained from all subjects.

Patients with OAG had gonioscopically open angles and fulfilled at least two of the following three criteria: history of intraocular pressure (IOP) above 21 mmHg, characteristic nerve fiber bundle visual field defects and glaucomatous optic disc changes. Ocular hypertensives had a history of IOP above 24 mmHg on at least two occasions, normal visual field and an ONH appearance that ranged from normal to suspect but showed no localized thinning, saucerization, notching or progression. Normal subjects had IOP below 21 mmHg, normal visual field and normal appearance of ONH with no characteristics suspicious of glaucomatous optic neuropathy. They also had normal ocular examinations and no family history of glaucoma. OAG and OHT patients were considered uncontrolled when their current IOP was higher than their target IOP and therefore considered candidates for further therapeutic intervention.

Measurements were performed on one eye of each subject. The study eye was chosen based on media clarity, larger neuroretinal rim area and better fixation with the fellow eye. When there was no difference between eyes in these criteria, one eye was selected randomly. Subjects were excluded from the study if they had other abnormal ocular

findings apart from pseudophakia, if significant media opacities or poor tear film quality precluded SLDF imaging, or if at any time they were unable to cooperate.

For each patient, medical and ocular history was taken. Intraocular pressures, best-corrected visual acuity and refractive errors were measured. A routine ophthalmologic examination including biomicroscopy, gonioscopy and ophthalmoscopy was performed and a recent automated perimetry (Humphrey Field Analyzer, program 24-2 Humphrey Instruments, San Leandro, California) was used to evaluate the visual field. Systemic blood pressure and heart rate were recorded and ocular perfusion pressure (OPP) was calculated according to the formula:

OPP = 2/3 [Diastolic Blood Pressure + 1/3 (Systolic Blood Pressure - Diastolic Blood Pressure)] – IOP.

The scanning laser Doppler flowmeter used in this study [Heidelberg Retina Flowmeter (HRF), Heidelberg Engineering, GmbH, Heidelberg, Germany] is a noninvasive instrument combining both a laser Doppler flowmeter with a scanning laser technique. It measures the amount of backscattered light at different locations in the tissue of interest in a short period of time. Detailed descriptions of the instrument and measurement techniques have been previously published.[20, 21] An infrared diode laser with wavelength 780 nm and a power of 200 μW is used. The area examined measures 2.7 mm x 0.7 mm in size and is composed of 64 horizontal lines, each with 256 points giving an approximate spatial resolution of 10 μm. Each line is scanned sequentially 128 times with a total acquisition time of 2.05 seconds. A two dimensional map of microvascular perfusion of the area to be studied is thus generated.

New scanning laser Doppler flowmetry analysis software [SLDF version 3.3] developed by Michelson and associates[19] enhances the computations generated by the HRF. The software excludes pixels with incorrect brightness (invalid pixels), marks saccades that lead to erroneous perfusion data and eliminates pixels of retinal vessels with a diameter greater than 30 μm. The analysis is based on the average of all valid image points and the perfusion map is divided into neuroretinal rim area, temporal peripapillary retinal area and nasal peripapillary retinal area. Each area is analyzed separately. Pupils 3 mm in diameter or smaller were dilated using tropicamide 1% (Alcon, Fort Worth, Texas). The fundus camera was adjusted until a focused, evenly illuminated and centered view of the ONH was obtained. The patient was asked to use the fellow eye for fixation and to refrain from movement and blinking during image acquisition. Using a 2.5-degree x 10- degree frame, a total of 7-9 images were then acquired in one session, focussing on the superficial retina. The angulation of the fundus camera as well as its distance from the eye was kept constant throughout the imaging session. All images were reviewed by the same observer and prior to data analysis the best five images in terms of focussing, centration, brightness and absence of movements were chosen. Patients whose images were considered to be of poor quality or unsuitable for analysis were excluded from the study. Full-field perfusion analysis was then performed on each of the five chosen images and mean values of blood flow for the neuroretinal rim of the ONH, the temporal peripapillary retina and the nasal peripapillary retina were obtained in arbitrary units (au).

Mean flow measurements were first transformed (square root transformation) to normalize distributions. One-way analysis of variance (ANOVA) was then used to compare mean values of the three study groups (OAG, OHT and NOR). Statistical significance was set at $P < 0.05$. Significant analyses were followed by Sidak multiple comparison tests to locate differences. Flow values of the neuroretinal rim of the ONH, nasal peripapillary retina and temporal peripapillary retina were then correlated with the clinical parameters of age, error of refraction, C/D ratio, IOP, visual field mean defect, maximum-recorded IOP and ocular perfusion pressure using Pearson's correlation. Statistical significance was set at $P < 0.01$.

The position of the surface of the neuroretinal rim relative to the dominant focal plane, the peripapillary retina, may be different in each of the three study groups and this difference may affect perfusion results. We therefore used the tomography capability of the combined Heidelberg retinal flowmeter/tomograph to retrospectively evaluate the magnitude and significance of these positions. Mean topographies were calculated from three high quality Heidelberg Retina Tomograph (HRT, Heidelberg Engineering, Heidelberg, Germany) images of the ONH and peripapillary retina, obtained at the time of the SLDF session based on techniques previously described[22-26] and using software version 2.01. When changes in the position of the reference plane were accounted for, the location of the rim is determined by its average Z coordinate (i.e. its average location relative to the mean peripapillary retinal surface height) as described in our previous study.[27] A positive value of the mean rim Z coordinate means that, on average,

the rim surface is located posterior to the peripapillary retinal surface i.e. defocused posteriorly, while a negative value of the mean rim Z coordinate means that, on average, the rim surface is located anterior to the peripapillary retinal surface i.e. defocused anteriorly.

RESULTS

SLDF imaging was performed on 32 patients with OAG, 24 patients with OHT and 29 normal subjects. Good quality images were obtained from 22 OAG patients, 20 OHT patients and 21 normal subjects and were analyzed. From these, two OAG patient and one normal subject were excluded for invalid rim data. Data is thus presented for 20 OAG patients, 20 OHT patients and 20 normal subjects. Causes of poor quality images were primarily excessive eye movements and media opacities. Invalid rim data was a term generated by the software in cases where the extremely low reflectivity of the ONH, sometimes accompanied by high C/D ratio, caused inadequate number of pixels in the neuroretinal rim area to be available for analysis. The two OAG patients excluded for invalid rim data had C/D ratio > 0.8 whereas the normal subject (with C/D ratio of 0.4) had an unusually low reflectivity of the neuroretinal rim. All three subjects were excluded by the software.

Table 4.1 summarizes the characteristics of the three study groups. Statistically significant differences were demonstrated between the groups in clinical C/D ratio, IOP and visual field mean defect.

Six OAG patients (30%), four OHT patients (20%) and two normal subjects (10%) had systemic hypertension. Two OAG patients (10%), one OHT patient (5%) and one normal subject (5%) had diabetes mellitus. Patients were allowed to continue on their prescribed systemic medications as well as their topical antiglaucoma medications. At the time of imaging, six of the twenty patients (30%) in the OAG group were on

systemic therapy [ACE inhibitors, 4 (20%); beta-blockers, 1 (5%) and diuretics, 1 (5%)]. Five of the twenty patients (25%) in the OHT group were on systemic therapy [ACE inhibitors, 2 (10%); anticoagulants, 1 (5%) and diuretics, 2 (10%)]. Two of the twenty patients (10%) in the NOR group were on systemic therapy [beta-blockers]. Seventeen of the twenty patients (85%) in the OAG group and eleven of the twenty patients (55%) in the OHT group were on topical antiglaucoma medications whether monotherapy or combinations [OAG: beta-blockers, 15 (75%) alpha-adrenergics, 5 (25%); cholinergics, 4 (20%); CAIs, 10 (50%)]. [OHT: beta-blockers, 10 (50%); alpha-adrenergics, 2 (10%); cholinergics, 1 (5%)]. Three patients (15%) in the OAG group had previous laser trabeculoplasty in the study eye. One OAG patient (5%) had a remote combined cataract and glaucoma surgery and five normal subjects (25%) had a remote cataract surgery in the study eye.

Table 4.2 and Figure 4.1 summarize the SLDF blood flow measurements (Mean ± SD) for each study group. OAG patients demonstrated significantly lower blood flow values in the neuroretinal rim compared to OHT patients (-43%) and to normal subjects (-37%) [P = 0.001, one-way ANOVA]. No statistically significant difference in flow values was observed in the neuroretinal rim between the OHT group and the NOR group [P = 0.91, Student's t-test]. Furthermore, no statistically significant difference in flow values was observed between the three groups in temporal and nasal peripapillary retina [P = 0.93 and 0.76 respectively, one-way ANOVA].

Three age-matched subgroups of 15 OAG, 15 OHT and 15 NOR subjects were then compared to exclude the influence of age on our results. In these subgroups, OAG patients had a mean (± SD) age of 63 ± 10 years, OHT patients had a mean age of 61 ± 11 years and NOR subjects had a mean age of 63 ± 11 years. In the OAG subgroup mean rim flow was 143 ± 85 au. Conversely, the OHT subgroup had a mean rim flow of 313 ± 161 au, [P = 0.0017, Student's t-test] while the NOR subgroup had a mean rim flow of 280 ± 101 au, [P = 0.0004, Student's t-test]. Peripapillary retinal flow showed no significant difference between groups [$P \geq 0.577$].

We also matched eight OAG patients and eight OHT subjects for IOP. Both groups had a mean (± SD) IOP of 26 ± 3 mmHg. In the OAG subgroup mean rim flow was 126 ± 73 au while in the OHT subgroup mean rim flow was 300 ± 222 au, [P = 0.066, Student's t-test]. Temporal and nasal peripapillary retinal blood flow showed no significant difference between the two subgroups [P = 0.505 and 0.251 respectively]. Similarly, eight OAG patients and eight NOR subjects were matched for IOP. Both groups had IOP of 18 ± 2 mmHg. In the OAG subgroup mean rim flow was 188 ± 88 au while in the NOR subgroup mean rim flow was 341 ± 90 au [P = 0.004, Student's t-test]. Temporal and nasal peripapillary retinal blood flow showed no significant difference between the subgroups [P = 0.058 and 0.825 respectively].

The decreased mean rim flow in the OAG subgroups compared to each of the OHT and NOR subgroups show that neither age nor IOP have influenced the observed perfusion difference between the three study groups.

A correlation matrix was then established for our study population (N = 60) between blood flow values of the neuroretinal rim, nasal and temporal peripapillary retina versus the clinical parameters age, C/D ratio, IOP, visual field mean defect, maximum-recorded IOP and ocular perfusion pressure (Table 4.3). Cup/disc ratio was the only parameter that showed a significant inverse correlation with neuroretinal rim blood flow [$r = -0.415$, $P = 0.001$, Pearson's correlation]. Neuroretinal rim blood flow showed no such correlation with the other parameters examined [$P \geq 0.13$]. Peripapillary retinal blood flow showed no significant correlation with any clinical parameter [$P \geq 0.14$].

Correlation between neuroretinal rim flow and these parameters was then calculated for each of the three study groups to test if such a correlation was present within each group, but the sample size was too small to show any significant correlation. However, there was an inverse but statistically non-significant relationship between neuroretinal rim blood flow and C/D ratio in the OHT group [$r = -0.363$, $P = 0.115$, Pearson's correlation].

In order to better understand the relationship between C/D ratio and neuroretinal rim blood flow in the OHT group, we split the OHT group into two equal subgroups: Ten subjects having C/D ratio equal to or greater than 0.4 (mean 0.54 ± 0.13) and 10 subjects having C/D ratio less than 0.4 (mean 0.27 ± 0.07). OHT eyes with C/D ratio $\geq$ 0.4 (N = 10) showed significantly lower mean neuroretinal rim flow compared to OHT

eyes with C/D ratio < 0.4 (N = 10) [203 ± 79 au versus 350 ± 185 au, P = 0.039, Student's t-test] (Figure 4.2).

OHT eyes with C/D ratio ≥ 0.4 showed significantly lower mean neuroretinal rim flow compared to the NOR group (203 ± 79 au versus 272 ± 93 au, P = 0.047) whereas no statistically significant difference was shown compared to the OAG group (203 ± 79 au versus 158 ± 79 au, P = 0.156). On the other hand, OHT eyes with C/D ratio < 0.4 showed significantly higher mean neuroretinal rim flow compared to the OAG group (350 ± 185 au versus 158 ± 79 au, P = 0.010) whereas no statistically significant difference was shown compared to the NOR group (350 ± 185 au versus 272 ± 93 au, P = 0.232).

Proper interpretation of SLDF perfusion images requires optimized technical settings specifically as regards reflectivity and focusing. Differences in reflectivity and focusing among study groups may introduce bias in analysis especially if thinner rim/larger cup is related to incorrect brightness and/or differences in focusing.

SLDF full-field perfusion analysis software[19] assumes adequate brightness. In order to meet this requirement, pixels with incorrect brightness i.e. underexposed and overexposed pixels, are excluded from analysis. Differences in percentage of eliminated pixels among groups may introduce bias in the analysis. Therefore it is important to know the percentage of pixels used by the software in each study group and whether there were differences among groups with respect to these percentages.

Compared with the total number of pixels in the scan area, the percentage of neuroretinal rim area pixels was significantly different between the three study groups (12.7% ± 6.3% in the OAG group, 23.5% ± 7.4% in the OHT group and the 30.4% ± 6.2% in the NOR group)[$P < 0.0001$ one-way ANOVA]. This is a clear consequence of the OAG group having smaller rim compared to the other groups. However, the percentage of valid pixels in the rim area (i.e. pixels used by the software for perfusion analysis) showed little difference among the three study groups (15.1% ± 17.0% in the OAG group, 18.0% ± 19.4% in the OHT group and the 18.7% ± 11.1% in the NOR group). This difference was not statistically significant [$P = 0.681$, one-way ANOVA]. In order to determine whether there was a relationship between the number of valid rim pixels and the rim flow values in glaucoma patients, we performed a comparison between neuroretinal rim blood flow in glaucoma patients with larger (upper third, N=7) number of valid pixels versus smaller (lower third, N=7) number of valid pixels. We found no significant difference in neuroretinal rim blood flow between these two subgroups ($P = 0.47$, Student's T-test). Decreased neuroretinal rim blood flow in glaucoma patients was not related to the number or percentage of valid pixels used for perfusion analysis.

Differences in mean rim height relative to the peripapillary retina were evaluated in each of the three study groups. Both the OAG and the OHT groups demonstrated a mean posterior displacement in the neuroretinal rim surface of 104 ± 94 μm and 64 ±

119 μm respectively whereas the NOR group demonstrated an anterior displacement of 23 ± 89 μm [P = 0.021, one-way ANOVA].

Table 4.1: Patient Characteristics of the three study groups (Mean ± SD, P value)

	OAG GROUP (N = 20)	OHT GROUP (N = 20)	NOR GROUP (N = 20)	Significance P < 0.05
Age (Yrs)	66.6 ± 10.8	57.2 ± 11.6	63.7 ± 9.8	0.07[ə]
Sex (M/F)	9/11	9/11	9/11	
IOP	22.2 ± 4.2	28.7 ± 3.9	16.9 ± 2.6	<0.0001[ə]
IOP Max	28.8 ± 6.2	30.3 ± 3.3	-	0.15[‡]
C/D ratio	0.75 ± 0.2	0.41 ± 0.2	0.23 ± 0.1	<0.0001[ə]
M.D.	-9.94 ± 8.25	-0.38 ± 2.4	-	0.00[‡]
OPP	43.2 ± 6.1	42.8 ± 10.6	48.2 ± 7.2	0.08[ə]

OAG = open angle glaucoma; OHT = ocular hypertension; NOR = normal; IOP = intraocular pressure (mmHg); IOP Max = maximum recorded intraocular pressure (mmHg); C/D ratio = cup to disc ratio, M.D. = mean defect of visual field; OPP = calculated ocular perfusion pressure.

One-way ANOVA and Sidak multiple comparisons[ə]

Non-parametric Analysis: (Mann-Whitney U test)[‡]

Table 4.2: SLDF Flow Measurements of the neuroretinal rim of ONH, temporal peripapillary retina and nasal peripapillary retina of the OAG, OHT and NOR groups.

LOCATION	OAG Group N=20	OHT Group N=20	% Diff. OAG vs OHT	NOR Group N=20	% Diff. OAG vs NOR	P Value
Neuroretinal rim of ONH	157 ± 78	276 ± 157	-43%	272 ± 92	-37%	0.001
Temp. Peripapillary retina	316 ± 83	309 ± 78	2%	307 ± 85.5	3%	0.93
Nasal Peripapillary retina	303 ± 104	287 ± 104	6%	279 ± 79	9%	0.76

OAG = open angle glaucoma; OHT = ocular hypertension; NOR = normal; ONH = optic nerve head; % Diff. = percentage difference

One-way ANOVA at 0.05 level

Table 4.3: Correlation between RIM, NAS and TEM blood flow versus various clinical parameters (N=60).

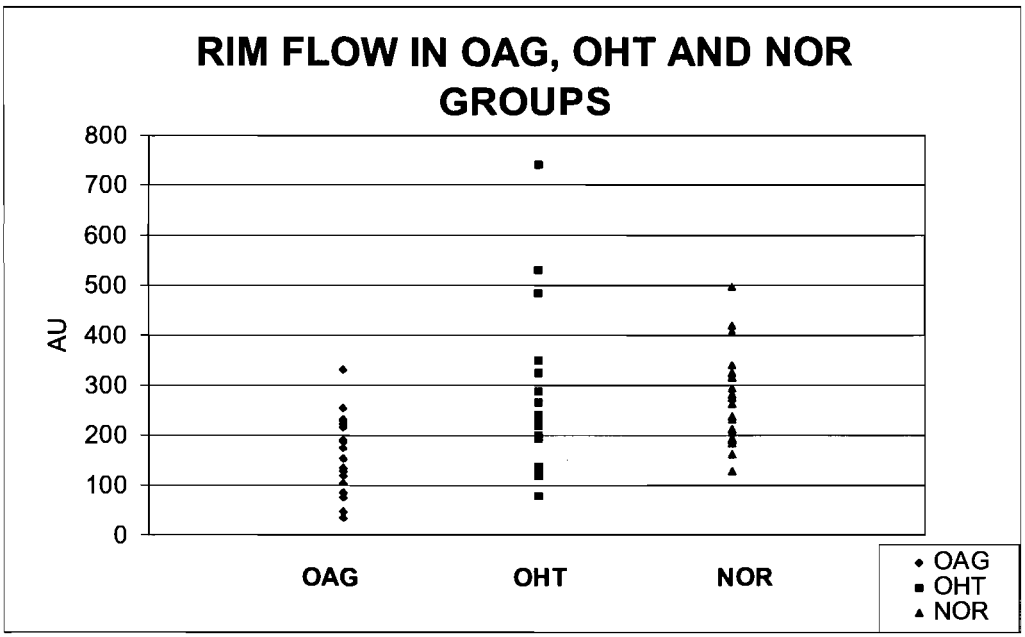
Parameter	Neuroretinal Rim of ONH		Nasal Peripapillary Retina		Temp Peripapillary Retina	
	R value	P value	R value	P value	R value	P value
Age	0.138	0.29	0.069	0.60	-0.073	0.58
IOP	0.001	0.99	-0.075	0.57	-0.013	0.92
C/D ratio	-0.415	0.00	0.192	0.14	0.124	0.35
M.D.*	0.247	0.13	-0.193	0.24	-0.091	0.58
T Max*	0.153	0.36	-0.080	0.63	0.111	0.51
OPP	-0.010	0.94	0.131	0.32	-0.060	0.65

OAG = open angle glaucoma; OHT = ocular hypertension; NOR = normal; IOP = intraocular pressure (mmHg); IOP Max = maximum recorded intraocular pressure (mmHg); c/d = cup/disc ratio, M.D. = mean defect of visual field; OPP = calculated ocular perfusion pressure.

Pearson Correlation – Significance (two-tailed) at 0.01 level

* N = 40

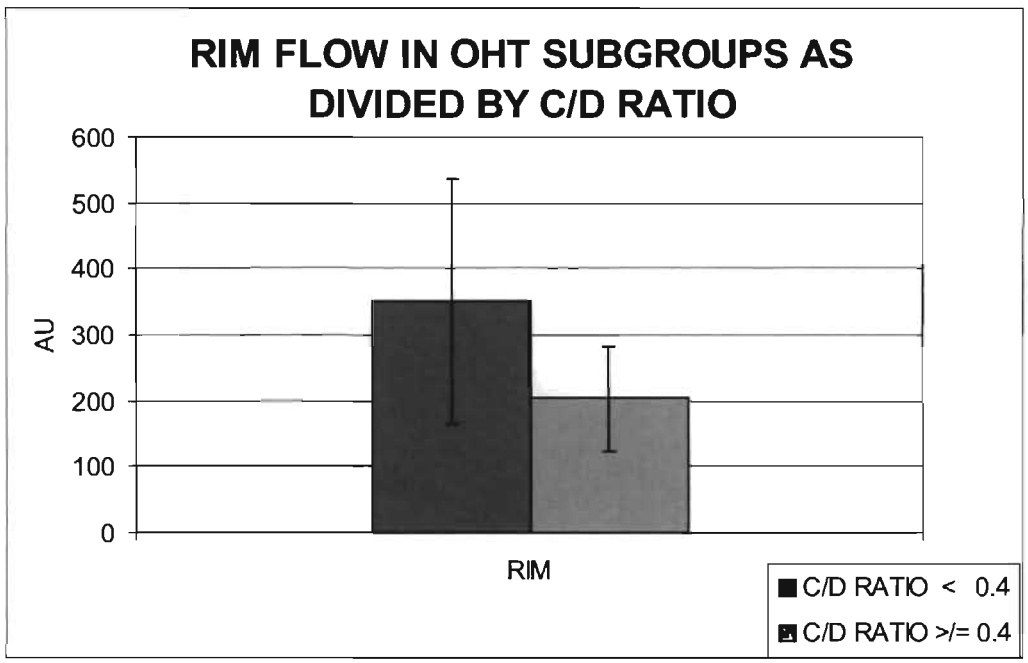
Figure 4.1.



Scattergrams of SLDF blood flow measurements for the neuroretinal rim of the ONH in the OAG group, OHT group and NOR group; One-way ANOVA at 0.05 level.

OAG = open angle glaucoma; OHT = ocular hypertension; NOR = normal; AU= arbitrary units

Figure 4.2.



SLDF blood flow measurements (Mean ± SD) for the neuroretinal rim of the ONH in the OHT group as divided into two equal subgroups based on C/D ratio; Student's T-Test at 0.05 level. RIM = neuroretinal rim of the ONH; OHT = ocular hypertension; C/D ratio = cup to disc ratio; AU = arbitrary units.

DISCUSSION

This study demonstrates that, as measured with scanning laser Doppler flowmetry and full-field perfusion analysis, OAG patients have significantly lower blood flow in the neuroretinal rim of the ONH when compared to OHT patients and normal subjects. Peripapillary retinal blood flow did not show such a difference between the three study groups. The reduction in neuroretinal rim blood flow was significantly correlated to the increasing C/D ratio.

Our results confirm the findings of several studies that reported a significantly reduced retro-ocular and bulk choroidal blood flow and increased vascular resistance in OAG compared to normal subjects and ocular hypertensives.[28-42] Using fluorescein angiography, Wolf and associates[43] demonstrated that eyes with OAG are associated with an increased arteriovenous passage time and a decreased dye velocity. Using single-point laser Doppler flowmetry, several authors similarly reported decreased blood flow in the ONH of OAG when compared to control subjects[44] and to glaucoma suspects.[45]

SLDF has been used in several studies comparing flow measurements of OAG patients and normal subjects. Michelson and associates[46] reported that both neuroretinal rim blood flow and peripapillary retinal blood flow were significantly decreased in OAG patients compared to age-matched controls. Nicolela et al[47] reported a significant decrease in blood flow in the lamina cribrosa and the upper temporal but not the lower

temporal peripapillary retina in OAG patients compared to control subjects. No difference in flow measurements of the neuroretinal rim was found. Findl and associates, [48] using both SLDF and fundus pulsation amplitudes, similarly reported reduced blood flow in the disc cup (-46%) and the neuroretinal rim (-18%) in patients with OAG when compared to age-matched control subjects. On the other hand, Hollo and associates failed to detect significant differences in neuroretinal rim blood flow[49] or in peripapillary retinal blood flow[50] in their population of open-angle glaucoma patients and normal tension glaucoma patients compared to control subjects.

SLDF has also been used to compare ONH and retinal perfusion of OAG patients and ocular hypertensives. Kerr and associates[51] reported reduced blood flow in the lamina cribrosa and the temporal neuroretinal rim of the ONH of glaucoma patients in comparison to ocular hypertensives. However no difference was found between groups at the nasal neuroretinal rim or nasal peripapillary retina and an increase in minimum velocity was reported at the temporal peripapillary retina in the glaucoma group.

Our results confirm the findings of Michelson and associates[46] as regards reduced blood flow in the neuroretinal rim of glaucoma patients compared to controls and those of Kerr and associates[51] as regards reduced blood flow in the neuroretinal rim of glaucoma patients compared to ocular hypertensives. However, we could not demonstrate significant differences in ONH blood flow between OHT group and NOR group. For peripapillary retinal blood flow, no significant difference was seen between the three study groups. We believe that the reason for such discrepancies might be that all the

reported studies used for analysis the conventional evaluation of 10x10 pixels measurement box which was reported to show several technical limitations including: [52]

- Variation in location of the measurement box selected by the operator between images. The slightest difference in the location of the measurement box in relation to retinal or ONH vessels may lead to considerable variation of perfusion values between images.
- Placement of a large measurement box on the neuroretinal rim of glaucoma patients with high C/D ratio and thin rim unavoidably includes an area of the cup or the peripapillary retina in the box thus leading to erroneous data.
- The appropriate illumination of the ocular tissue during image acquisition taking into consideration the low reflectivity of the ONH compared to the peripapillary retina and the influence of brightness on the measurements of the SLDF.
- Differences in focusing between the neuroretinal rim and peripapillary retina versus the lamina cribrosa. Different focal planes should be used for evaluation of cup and rim perfusion.

Using the 10x10 pixel measurement box, reproducibility of the neuroretinal rim flow was considered poor with an intersession coefficient of reliability of 0.36 in glaucoma patients and 0.47 in normal volunteers[53] although peripapillary retinal flow appeared to be more reproducible.[27, 53,54]

In the present study we utilized the SLDF full-field perfusion analysis software (version 3.3). The software was reported to provide higher reproducibility values than those

reported using the 10x10 pixels measurement box.[25] Iester et al[55], using the same software, reported good intraobserver reproducibility (intra-image and inter-image). However the reproducibility was still significantly better in the peripapillary retina than in the rim area. We have demonstrated that obtaining mean values from five high-quality images further improves the reproducibility of the technique and were able to report an *intrasession* coefficient of reliability that ranged from 0.93-0.99 and an *intersession* coefficient of reliability that ranged from 0.80-0.99 in our population of glaucoma patients and normal volunteers (Bizzarro et al, Invest Ophthalmol Vis Sci 2001: 42; S21).

This study is also unique in its examination of all three pertinent groups, OAG, OHT and normals. Our study groups included uncontrolled OAG and OHT patients, some of whom were newly diagnosed receiving no treatment and others were uncontrolled despite topical antiglaucoma medications. The heterogeneity of the medications and the combined therapies gave very small groups, which precluded statistical evaluations, however no specific trend was apparent. Comparisons of blood flow values between patients using antiglaucoma medications and patients not on therapy could not be performed because the sample size was too small.

We observed an inverse correlation between neuroretinal rim flow values and C/D ratio [-0.415, P = 0.001], which is suggestive of a link between defective perfusion of the ONH and severity of glaucomatous optic disc changes. The correlation with C/D ratio

was across all three groups and in contrast there was no similar correlation between neuroretinal rim flow values and visual field mean defect suggesting that rim blood flow was correlated with C/D ratio rather than with patient's diagnosis (or perimetric status). A similar correlation between ONH blood flow and C/D ratio was reported by Michelson et al in glaucoma patients.[46]

The OHT group had a range of C/D ratios from 0.15 to 0.70 (mean 0.41 ± 0.2). Mean neuroretinal rim blood flow was lower in OHT patients with larger C/D ratios when compared to OHT patients with smaller C/D ratios [P = 0.039, Student's t-test] (Figure 4.2). A similar correlation was reported by Piltz-Seymour and associates[45] in their evaluation of optic nerve head perfusion in glaucoma suspects using single-point laser Doppler flowmetry.

Changes in focus might artifactually change SLDF measurements. Perfused tissue located significantly anterior or posterior to the focal plane has been shown to yield artifactually *higher* SLDF perfusion values. In recent studies, which we have confirmed in our laboratory (data not shown), (Lundmark et al, Invest Ophthalmol Vis Sci 37: S265, 1996 and Segawa et al, Invest Ophthalmol Vis Sci 38: S774, 1997) it was shown that as the focal plane is moved either anterior or posterior to the surface of rim tissue, measured values artifactually increase. Prokopich et al [Invest Ophthalmol Vis Sci 37: S265, 1996] reported that a focal plane displacement of 200 μm (0.5 dioptres) was found to result in an artifactual change in perfusion of 30 au.

We have therefore meticulously established the optimal focal plane during our imaging sessions using the tomography capability of the combined flowmeter/tomograph. We have also retrospectively evaluated the position of the surface of the neuroretinal rim with respect to the dominant focal plane, the surface of the peripapillary retina, in each of the three study groups. Since most OAG patients and some OHT patients show anteroposterior thinning of the neuroretinal rim, one would expect that the rim surface would commonly be located posterior to the surface of the peripapillary retina. Our analysis demonstrates a posterior defocusing of the rim relative to the mean peripapillary retinal surface of 104 μm in the OAG group and 64 μm in the OHT group, while the NOR group demonstrates an anterior defocusing of 23 μm. This posterior defocusing of the neuroretinal rim in the OAG group would have manifested as an increase (rather than a decrease) in rim perfusion values in the range of 15 au, which is considered too small to have significantly altered our findings.

In conclusion, we have demonstrated defective perfusion in the neuroretinal rim of the ONH in glaucoma patients when compared to ocular hypertensives and normal subjects, as measured by SLDF full-field perfusion analysis. We were not able to attribute such defective perfusion to the use of topical or systemic medications. It was also not established whether these changes precede or result from glaucomatous optic disc changes. A definite inverse correlation between reduced neuroretinal rim flow values and higher C/D ratio was also established. This correlation was apparent, though not significant, within the OHT group, suggesting that neuroretinal rim perfusion may be

reduced in high-risk ocular hypertensives prior to the manifestation of visual field defects.

We believe our data may set the groundwork for a long-term study to examine whether OHT patients with lower rim perfusion values are more likely to show progression to glaucoma, whether we can distinguish between OHT patients needing treatment versus those that do not based on rim perfusion values, and, finally, whether ONH perfusion might be a prognostic marker for future stability in both OAG and OHT patients. This will eventually lead to better understanding of the disease and better therapy for our patients.

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Chapter 5

Changes in Optic Nerve Head Blood Flow after Therapeutic Intraocular Pressure Reduction in Glaucoma Patients and Ocular Hypertensives.

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PRECIS

Following therapeutic intraocular pressure reduction, patients with open angle glaucoma show marked improvement of optic nerve head blood flow as measured by scanning laser Doppler flowmetry compared to ocular hypertensives.

ABSTRACT

Purpose: To detect and quantify changes in optic nerve head (ONH) and peripapillary retinal blood flow by scanning laser Doppler flowmetry (SLDF) in open angle glaucoma (OAG) and ocular hypertension (OHT) following therapeutic intraocular pressure (IOP) reduction.

Design: Prospective, nonrandomized, self-controlled trial.

Participants: Twenty patients with OAG and twenty patients with OHT with clinical indication for therapeutic IOP reduction were prospectively enrolled.

Intervention: IOP reduction was achieved by medical, laser or surgical therapy. All patients had IOP reductions more than 20% and a minimum of four weeks follow-up.

Main Outcome Measures: Blood flow measurements were performed by SLDF analysis software (version 3.3) using Heidelberg Retina Flowmeter images. Statistical evaluations were performed on both groups using a two-tailed distribution paired t-test.

Results: Twenty patients with OAG had a mean IOP reduction of 37% after treatment. In these patients mean (±SD) rim blood flow increased by 67% [from 158±79au to 264±127au, p=0.001], while mean temporal peripapillary retinal flow decreased by 7.4% [p=0.24] and mean nasal peripapillary retinal flow increased by 0.3% [p=0.96]. Twenty OHT patients had a mean IOP reduction of 33% after treatment. In contrast to the OAG group, neither the mean rim blood flow [7.5% increase from 277±158au to 298±140au, p=0.41] nor the mean temporal [p=0.35] or nasal [p=0.88] peripapillary retinal flow changed significantly.

Conclusion: For a similar percentage of IOP reduction, OAG patients had a statistically significant improvement of blood flow in the neuroretinal rim of the ONH whereas OHT patients did not demonstrate such a change. Peripapillary retinal blood flow, expected to be less affected in glaucoma, remained stable in both groups. In addition to indicating a response to therapy in OAG patients, the reported changes in rim perfusion suggest that ONH autoregulation may be defective in OAG while intact in OHT.

INTRODUCTION

The pathogenesis of optic nerve damage in glaucoma is still not fully understood. There is substantial evidence indicating that glaucomatous optic neuropathy is multifactorial in nature with elevated intraocular pressure (IOP) being the most common risk factor. However, vascular factors have been postulated to play a major role.[1-3] These factors include autoregulation of blood flow in the optic nerve head (ONH) and other ocular tissues, local vasospasm, arterial hypertension and nocturnal hypotension.

Although such vascular factors have been studied decades ago, only recent technical developments have enabled non-invasive investigations of the associated circulatory disturbances. These investigations point to defective ONH blood flow as a likely contributing factor in the development of glaucomatous optic neuropathy.[2,3] Consequently, it could be assumed that ONH blood flow might improve following institution of therapy that would control the IOP and stabilize the glaucomatous optic neuropathy.

Optic nerve head blood flow depends upon ocular perfusion pressure, which can be defined as the mean arterial blood pressure in the ocular vessels minus the intraocular pressure. Thus, in the absence of autoregulation, there is an inverse relationship between IOP and ocular perfusion pressure. The higher the IOP the lower the ocular perfusion pressure and consequently the lower the blood flow in the ONH. On the other

hand, reduction of IOP would be expected to improve ocular perfusion pressure and consequently increase ONH blood flow.[4]

The purpose of autoregulation in the ONH is to maintain a relatively constant blood flow in spite of changes in ocular perfusion pressure. The existence of intact autoregulation in the normal ONH has been demonstrated in a large number of experimental[5-9] and clinical[10-13] studies.

Autoregulation is reported to operate only within a critical range of ocular perfusion pressure and becomes ineffective when the ocular perfusion pressure goes below or above this critical range.[4] This range of ocular perfusion pressure has been investigated in different species using various methodologies[5-7,14-17.] Geijer and Bill[5] reported autoregulation of the ONH to be normal at an ocular perfusion pressure of >30 mmHg. Ernest[14] reported similar findings with pressures >50 mmHg. Breakdown of autoregulation was reported to take place at <30 mmHg by Bill and Sperber[15], at <25 mmHg by Sossi and Andersen[6] and at 30-35 mmHg perfusion pressure by Hayreh and coworkers.[16]

It is also hypothesized that glaucomatous optic neuropathy maybe due to an eventual breakdown in ONH autoregulation[10,11,18,19.] If this hypothesis was correct then similar changes in IOP, while accompanied by changes in ONH blood flow in glaucoma patients, would not be associated with such changes in subjects lacking glaucomatous

optic neuropathy. We set out to test this hypothesis on patients with open angle glaucoma (OAG) and ocular hypertension (OHT). Our study was performed in a true clinical context, on patients that required therapeutic IOP reductions.

We performed our measurements using scanning laser Doppler flowmetry (SLDF). This system permits direct quantitative measurements of ONH and peripapillary retinal perfusion[20,21,25]. It has been reported to give both valid[20-22,26,27] and reproducible[20,21,23-25] results.

The aim of this study is to detect and quantify changes in ONH and peripapillary retinal blood flow using SLDF full-field perfusion analysis in patients with OAG and OHT undergoing therapeutic IOP reduction by medical, laser or surgical intervention.

PATIENTS AND METHODS

The study was approved by the research committee of Maisonneuve-Rosemont Hospital, University of Montreal. An informed consent was obtained from each patient before enrollment in the study.

Twenty patients with OAG and twenty patients with OHT were recruited from the glaucoma clinics of the hospital into this prospective study. Only patients achieving a minimum of 20% IOP reduction were eligible to complete the study.

Patients with OAG had glaucomatous optic neuropathy, characteristic nerve fiber bundle visual field defects and gonioscopically open angles with no restrictions for IOP. Ocular hypertensives had a history of repeated IOPs greater than 24 mmHg with normal visual fields and normal or suspect ONH appearance. Subjects were excluded from the study if they had abnormal ocular findings other than pseudophakia, if they had significant media opacities precluding SLDF imaging, or if they were unable to co-operate.

During the prestudy visit of each patient, medical and ocular history was recorded. Intraocular pressures, best-corrected visual acuity and refractive errors were measured. A routine ophthalmologic examination including biomicroscopy, gonioscopy and ophthalmoscopy was performed. A recent automated perimetry (Humphrey Field Analyzer, Program 24-2, Humphrey Instruments, San Leandro, California) was used to

evaluate the visual field. Systemic arterial blood pressure and heart rate were recorded. Ocular perfusion pressure (OPP) was calculated according to the formula: [4]

OPP = 2/3 [diastolic blood pressure + 1/3 (systolic blood pressure - diastolic Blood Pressure)] – IOP.

Statistical analysis of patients' characteristics and perfusion parameters was performed using two-tailed Student's t-test ($p < 0.05$).

The SLDF used in this study [Heidelberg Retina Flowmeter (HRF), Heidelberg Engineering, GmbH, Heidelberg, Germany] is a noninvasive instrument combining both a laser Doppler flowmeter with a scanning laser technique. It measures the amount of backscattered light at different locations in the tissue of interest in a short period of time. An infrared diode laser with wavelength 780 nm is used. The area examined measures 2.7 mm x 0.7 mm in size and is composed of 64 horizontal lines, each with 256 points giving an approximate spatial resolution of 10 μm. Each line is scanned sequentially a total of 128 times with a total acquisition time of 2.05 seconds. A two dimensional map of microvascular perfusion of the area to be studied is thus generated.

New SLDF analysis software [version 3.3] developed by Michelson and associates[25] enhances the computations generated by the HRF. The software excludes pixels with incorrect brightness, marks saccades that lead to erroneous perfusion data and eliminates pixels of retinal vessels with a diameter greater than 30 μm. The analysis is based on the average of all valid image points with the perfusion map divided into

neuroretinal rim area, temporal peripapillary retinal area and nasal peripapillary retinal area. Each area is analyzed separately (Figure 5.1).

We have recently demonstrated that SLDF full-field perfusion analysis produces highly reproducible intrasession and intersession measurements of ONH and peripapillary retinal blood flow in our population of glaucoma patients and normal volunteers (Bizzarro et al [Invest Ophthalmol Vis Sci 2000; 41(4): S556, 2000]). Using a mean of five images our intersession reproducibility in our glaucoma patients was 0.87 to 0.99, depending on the location of measurement.

Image Acquisition Technique

Pupils 3 mm in diameter or smaller were dilated. The fundus camera was adjusted until a focused, evenly illuminated and centered view of the ONH was obtained. The patient was asked to use the fellow eye for fixation and to refrain from movement and blinking during image acquisition. Using a 2.5-degree x 10-degree frame, a total number of 7-10 images were then acquired in one session, focussing on the superficial retina.

All images were reviewed by the same observer and prior to data analysis the best five images in terms of focussing, centration, brightness and absence of movements were chosen. Patients whose images were considered to be of poor quality or unsuitable for analysis were excluded from the study. SLDF full-field perfusion analysis was then performed on each of the five images with measurements for flow, volume and velocity given in arbitrary units (AU) for the neuroretinal rim area, temporal peripapillary retinal area and nasal peripapillary retinal area.

All patients then underwent IOP reduction by medical, laser, or surgical intervention. All patients included in the study had a sustained IOP reduction of 20% or more, as well as a minimum of four weeks follow up between the intervention and the second SLDF session.

Patients then underwent a second session of SLDF imaging using the same settings previously used for image acquisition (scan area, focussing, sensitivity) and full-field perfusion analysis (position of the rim circles, calculation parameters). The best five images were similarly chosen and analyzed. A mean of the five readings for the parameters flow, volume and velocity was then computed for each of the two sessions.

Statistical evaluations were performed on both OAG and OHT groups using two-tailed paired distribution t-test for the parameters flow, volume and velocity. Statistical significance was set at $P < 0.05$.

Perfused tissue located significantly anterior or posterior to the focal plane has been shown to yield artifactually higher SLDF values (Lundmark et al [Invest Ophthalmol Vis Sci 37: S265, 1996] and Segawa et al [Invest Ophthalmol Vis Sci 38: S774, 1997]). Since it has also been shown that ONH morphology changes following reduction of IOP,[28-30] we assessed the changes in the position of the surface of the neuroretinal rim relative to the dominant focal plane, the peripapillary retina, using confocal scanning laser ophthalmoscopy both before and after IOP reducton. Mean topographies were calculated from three high quality Heidelberg Retina Tomograph (HRT, Heidelberg

Engineering, Heidelberg, Germany) images of the ONH and peripapillary retina, obtained at the time of each of the two SLDF sessions based on techniques previously described[31-35] and using software version 2.01.

Mean rim height, the average height of the rim surface above the reference plane, was calculated using the equation:

Mean Rim Height = Volume Above Reference Plane / Rim Area (1)

where values for volume above reference plane and rim area are given by the HRT software in the tilted relative coordinate system.

When changes in the position of the reference plane were accounted for, the mean rim height would be its average Z coordinate (i.e. its average location relative to the mean peripapillary retinal surface height). This value can be calculated using the equation:

Mean Rim Z Coordinate = Reference Height – Mean Rim Height (2)

where values for the reference height are given by the HRT software and the mean rim height is calculated as described above.

The mean rim Z coordinate can be positive or negative. A positive value means that, on average, the rim surface is located posterior to the peripapillary retinal surface, while a negative value means that, on average, the rim surface is located anterior to the peripapillary retinal surface

RESULTS

Pre- and post-IOP reduction SLDF imaging was performed on 32 patients with OAG and 24 patients with OHT. Good quality images were obtained from 22 OAG patients and 20 OHT and were analyzed. From these, two OAG patients were excluded for invalid rim data. Data is thus presented for 20 OAG and 20 OHT patients.

Causes of poor quality images primarily were excessive eye movements and media opacities. Invalid rim data was the term generated by the software in two cases where the extremely low reflectivity of the ONH, accompanied by high cup/disc ratio, caused inadequate number of pixels in the rim area to be available for SLDF analysis.

The mean age (± SD) was 66.7±10.9 years for the OAG group (age range 42-79 years) and 57.2±11.6 years for the OHT group (age range 42-75 years). There were 9 males (45%) and 11 females (55%) in each of the two study groups.

Six OAG patients (30%) and four OHT patients (20%) had systemic hypertension. Two OAG patients (10%) and one OHT (5%) had diabetes mellitus. At the first imaging session, six of the twenty patients in the OAG group were on systemic therapy [ACE inhibitors, 4 (20%); beta-blockers, 1 (5%); diuretics, 1 (5%)]. Five of the twenty patients (25%) in the OHT group were on systemic therapy [ACE inhibitors, 2 (10%); anticoagulant, 1 (5%); diuretics, 2 (10%)].

Seventeen of the twenty patients in the OAG group were on topical glaucoma therapy, whether monotherapy or combinations [beta-blockers, 15 (75%); alpha-adrenergics, 5 (25%); cholinergics, 4 (20%); carbonic anhydrase inhibitors, 10 (50%)]. Eleven of the twenty patients (55%) in the OHT group were on topical glaucoma therapy (beta-blockers, 10 (50%); alpha-adrenergics, 2 (10%); cholinergics, 1 (5%). Three patients (15%) in the OAG group had a previous laser trabeculoplasty. One OAG patient (5%) had a remote combined cataract and glaucoma surgery.

Table 5.1 summarizes the characteristics of the OAG and OHT study groups. There was no statistically significant difference in the means of maximum-recorded IOP, refractive error and calculated ocular perfusion pressure between the two groups. Statistically significant differences were demonstrated between the OAG group and the OHT group in the means of age, IOP before reduction, clinical C/D ratio and visual field mean defect [$P \leq 0.01$].

The mean percentage of IOP reduction was 37% in the OAG group and 33% in the OHT group (Table 1). There were no statistically significant differences between the two groups in the percentage of IOP reduction and the follow-up duration [$p=0.35$ and 0.83 respectively]. A statistically significant difference was demonstrated between the OAG group and the OHT group in the mean IOP after reduction [$P = 0.0002$].

Therapeutic IOP reduction was attained in four of the twenty OAG patients by ocular hypotensive drugs, whether monotherapy or combinations [beta-blockers, 2 (10%); alpha-adrenergics, 1 (5%); cholinergics, 1 (5%) and CAIs, 2 (10%)]. Twelve of the twenty OHT patients had therapeutic IOP reduction by ocular hypotensive drugs [beta-blockers, 10 (50%); alpha-adrenergics, 2 (10%) and CAIs, 3 (15%)].

Table 5.2 summarizes the SLDF perfusion data (Mean ± SD, % change, p value) of the neuroretinal rim, temporal peripapillary retina and nasal peripapillary retina of both the OHT and OAG groups before and after IOP reduction. Prior to IOP-reduction, OAG patients demonstrated significantly lower blood flow, volume and velocity values in the neuroretinal rim area compared to OHT patients [$P \leq 0.005$]. No statistically significant difference in perfusion values was observed between OAG and OHT patients in the temporal and nasal peripapillary retina [$P \geq 0.79$ and $P \geq 0.63$ respectively].

Following sustained IOP reduction, in the OAG group (Figure 5.2 and Table 5.2) mean rim blood flow showed a statistically significant increase of 67.2% [$P = 0.001$]. Mean temporal peripapillary retinal flow decreased by 7.4% [$P = 0.24$] and mean nasal peripapillary retinal flow increased by 0.3% [$P = 0.96$]. In contrast, in the OHT group (Figure 5.3 and Table 5.2), none of the flow parameters changed significantly: mean rim blood flow increased by 7.5% [$P = 0.41$], mean temporal peripapillary flow decreased by 5.2% [$P = 0.35$] and mean nasal peripapillary flow decreased by 1.0% [$P = 0.88$].

Large increases in neuroretinal rim flow were measured in the OAG group regardless of the procedure used to reduce IOP. These changes did not always reach statistical significance due to small sample size in the sub-groups. In the medically treated patients (N=4), the mean increase in rim flow following IOP reduction was 95.0% [P = 0.13], in the laser-treated patients (N=10) it was 47.6% [P = 0.07], while in the surgically-treated patients (N=6) the mean increase was 85.5% [P = 0.03]. Peripapillary retinal flow showed no significant change irrespective of the procedure used [$P \geq 0.22$].

The two groups had disparate mean post-reduction IOPs. To examine whether this disparity influenced the results we matched nine pairs of subjects from the two study groups with identical IOP readings following treatment (Table 5.3). In these sub-groups, OAG patients had a mean (± SD) IOP of 17.89 ± 2.8 following a 23.7% reduction and OHT patients had a mean IOP of 17.89 ± 2.9 following a 28.3% reduction of IOP. In the OAG subgroup, mean rim flow increased by 54.4% (from 173.8 ± 65.6 to 268.3 ± 121.6, P = 0.061). Mean temporal peripapillary retinal flow decreased by 6.7% (from 287.3 ± 75.8 to 268.0 ± 63.0, P = 0.49) and mean nasal peripapillary retinal flow increased by 5.7% (from 257.2 ± 99.3 to 271.9 ± 97.5, P = 0.47). On the other hand, the OHT patients showed no significant change in either the mean rim flow which decreased by 2.6% (from 350.2 ± 203.3 to 341.1 ± 166.8, P = 0.83) or the temporal or nasal peripapillary retinal flow (-5.9%, P = 0.49 and –12.3%, P = 0.26 respectively). Therefore, we could not find evidence that the post-reduction IOP alone influenced the observed difference between the two groups.

We also examined the OAG group for the influence of achieving single-digit post-reduction IOP on neuroretinal rim and peripapillary retinal blood flow. A comparison between patients with lower IOP (<10 mmHg) and those with higher IOP (>10 mmHg) was performed. OAG patients with post-reduction IOP of <10 mmHg (N=6) showed an increase in rim flow of 65.6% (from 190.5 ± 86.0 to 315.4 ± 153.1, P = 0.06). Statistical significance was not achieved due to the small size of this group. Peripapillary temporal and nasal retinal flow showed no significant change (-9.4%, P = 0.39 and -1.1%, P = 0.92 respectively). On the other hand, OAG patients with post-reduction IOP of >10 mmHg (N=14) showed an increase in rim flow of 68.2% (from 143.8 ± 74.4 to 241.9 ± 112.7, P = 0.007). Peripapillary temporal and nasal retinal flow showed no significant change (-6.5%, P = 0.42 and +1.1%, P = 0.88 respectively). Therefore increases in neuroretinal rim blood flow were not restricted to patients showing lower IOPs attained after therapy.

There was a significant difference in age between our two study groups (P = 0.011, Table 5.1). To examine the influence of age on our results we compared two age-matched subgroups of 15 OAG and 15 OHT patients (Table 5.4). In these subgroups, OAG patients had a mean (±SD) age of 63 ± 10 years while OHT patients had a mean age of 62 ± 10 years. Following therapeutic IOP reduction, the OAG subgroup shows an 83% increase in mean rim flow (P = 0.002) while there was only 2% increase in mean rim flow in the OHT subgroup (P = 0.3). Mean peripapillary retinal flow had small non-significant changes in either group. Thus, age differences between the OAG group and

OHT group were found to have no impact on group comparisons and did not appear to affect our perfusion results.

Changes in mean rim height following therapeutic IOP reduction were evaluated in each of the two study groups in order to determine to what degree such changes may have contributed to measured variations in perfusion data. Changes in the mean rim Z coordinate as defined in the *methods* are shown in Table 5.5 Following IOP reduction, there was a mean posterior displacement of 7 μm in the neuroretinal rim surface relative to the peripapillary retina in the OAG group [P = 0.60]. In the OHT group there was a mean anterior displacement of 2 μm in the neuroretinal rim surface relative to the peripapillary retina [P = 0.85]. Considering that the SLDF is reported to measure flow to a depth of at least 300 μm, it is unlikely that these displacements had a significant impact on our perfusion results.

Table 5.1: Characteristics of OAG and OHT groups (Mean ± SD)

	Age Yrs	Sex M/F	IOP Pre	IOP Max	C/D	M.D.	Refr.	Perf. Pr.	Method of IOP Reduction			IOP Post	Mean % IOP Red'n	Duration (wks)
									Med.	Las.	Sur.			
OAG Eyes (n = 20)	66.7 ± 10.9	9/11	22.2 ± 4.2	28.8 ± 6.2	0.75 ± 0.2	-9.94 ± 8.3	-0.9 ± 2.7	43.2 ± 6.1	4	10	6	14.2 ± 4.7	36.9 ± 16.1	14.2 ± 6.1
OHT Eyes (n = 20)	57.2 ± 12.3	9/11	28.7 ± 3.9	29.8 ± 3.5	0.41 ± 0.2	-0.38 ± 2.4	+0.2 ± 2.8	42.8 ± 10.6	12	8	0	19.0 ± 2.5	32.7 ± 11.5	14.6 ± 6.3
T Test	0.011	-	0.000	0.359	0.000	0.000	0.186	0.894	-	-	-	0.000	0.35	0.83

OAG = open angle glaucoma; OHT = ocular hypertension; IOP Pre = intraocular pressure prior to reduction (mmHg); IOP Max = maximum recorded intraocular pressure (mmHg); C/D = cup/disc ratio, M.D. = mean defect; Refr. = error of refraction; Perf. Pr. = calculated ocular perfusion pressure; Med. = medical therapy; Las. = argon laser trabeculoplasty; Sur. = surgery (trabeculectomy); IOP Post = intraocular pressure after reduction (mmHg); Mean % Red'n= Mean percentage reduction of IOP; Duration (wks) = Mean number of weeks between reduction of IOP and second session of SLDF.

Table 5.2: SLDF Perfusion Measurements in OAG and OHT Groups (Mean ± SD).

Location	Prameter	OHT GROUP (N= 20)				OAG GROUP (N=20)			
		Pre IOP Red'n	Post IOP Red'n	% Ch.	P Value	Pre IOP Red'n	Post IOP Red'n	% Ch.	P Value
NEURORETINAL RIM	FLO	276.8 ± 157.8	297.6 ± 139.7	**+7.5**	0.41	157.8 ± 78.9	263.9 ± 126.7	**+67.2**	0.001
	VOL	20.3 ± 6.5	19.3 ± 4.9	**-4.9**	0.45	13.6 ± 4.2	15.4 ± 5.0	**+13.2**	0.09
	VEL	1.33 ± 0.58	1.34 ± 0.51	**+0.8**	0.90	0.85 ± 0.35	1.12 ± 0.48	**+31.8**	0.006
TEMPORAL PP RETINA	FLO	309.0 ± 78.0	293.0 ± 68.0	**-5.2**	0.35	316.9 ± 83.4	293.3 ± 70.9	**-7.4**	0.24
	VOL	20.5 ± 4.8	19.0 ± 4.6	**-7.3**	0.24	20.9 ± 5.9	18.3 ± 3.7	**-12.4**	0.06
	VEL	1.10 ± 0.27	1.04 ± 0.23	**-5.5**	0.33	1.14 ± 0.31	1.05 ± 0.25	**-7.9**	0.18
NASAL PP RETINA	FLO	287.1 ± 104.1	284.1 ± 79.9	**-1.0**	0.88	303.4 ± 104.6	304.3 ± 90.7	**+0.3**	0.96
	VOL	17.6 ± 4.5	16.9 ± 4.1	**-4.0**	0.50	18.7 ± 5.2	17.4 ± 3.4	**-7.0**	0.20
	VEL	1.04 ± 0.33	1.04 ± 0.25	**0.0**	0.90	1.14 ± 0.35	1.11 ± 0.31	**-2.6**	0.70

OHT = ocular hypertension; OAG = open angle glaucoma; TEMPORAL PP RETINA= temporal peripapillary retina; NASAL PP RETINA= nasal peripapillary retina, Pre IOP Red'n= perfusion values before IOP reduction; Post IOP Red'n= perfusion values after sustained IOP reduction; % Ch = percentage of change in perfusion; FLO = flow; VOL = volume; VEL = velocity.

Table 5.3. Characteristics of Post IOP-Matched Subgroups (Mean ± SD)

	Age Yrs	Sex M/F	IOP Pre	IOP Max	C/D	M.D.	Refr.	Perf. Pr.	Method of IOP Reduction			IOP Post	% IOP Red'n	Duration (wks)
									Med	Las.	Sur.			
OAG Eyes (n = 9)	66 ± 12	5/4	23.7 ± 4.4	30.0 ± 7.8	0.66 ± 0.2	-3.99 ± 7.9	+0.03 ± 1.4	40.4 ± 6.3	4	6	5	17.9 ± 2.8	25.5 ± 6.17	10.8 ± 5.9
OHT Eyes (n = 9)	59 ± 11	4/5	28.3 ± 4.7	30.0 ± 3.2	0.43 ± 0.2	-0.14 ± 3.2	-0.06 ± 2.6	43.7 ± 12.3	9	6	0	17.9 ± 2.9	35.5 ± 13.2	17.1 ± 6.1
T Test	0.226	-	0.046	0.798	0.035	0.020	0.933	0.486	-	-	-	1.000	0.056	0.042

OAG = open angle glaucoma; OHT = ocular hypertension; IOP Pre = intraocular pressure prior to reduction (mmHg); IOP Max = maximum recorded intraocular pressure (mmHg); C/D = cup/disc ratio, M.D. = mean defect; Refr. = error of refraction; Perf. Pr. = calculated ocular perfusion pressure; Med. = medical therapy; Las. = argon laser trabeculoplasty; Sur. = surgery (trabeculectomy); IOP Post = intraocular pressure after reduction (mmHg); Mean % Red'n= Mean percentage reduction of IOP; Duration (wks) = Mean number of weeks between reduction of IOP and second session of SLDF.

Table 5.4. Characteristics of Age-Matched Subgroups (Mean ± SD)

	Age Yrs	Sex M/F	IOP Pre	IOP Max	C/D	M.D.	Refr.	Perf. Pr.	Method of IOP Reduction			IOP Post	% IOP Red'n	Durat. (wks)
									Med	Las.	Sur.			
OAG Eyes (n = 15)	63 ± 10	7/8	21.7 ± 4.2	28.9 ± 6.6	0.75 ± 0.2	-9.50 ± 8.6	-1.5 ± 2.8	43.2 ± 6.4	2	7	0	14.1 ± 4.5	35.8 ± 15.8	13.7 ± 6.3
OHT Eyes (n = 15)	62 ± 10	7/8	28.7 ± 3.9	29.8 ± 3.5	0.36 ± 0.2	-0.54 ± 2.7	+1.0 ± 0.5	41.2 ± 10.8	4	5	0	19.3 ± 2.7	31.7 ± 11.7	15.1 ± 6.4
T Test	0.707	-	0.000	0.520	0.000	0.001	0.011	0.556	-	-	-	0.001	0.425	0.528

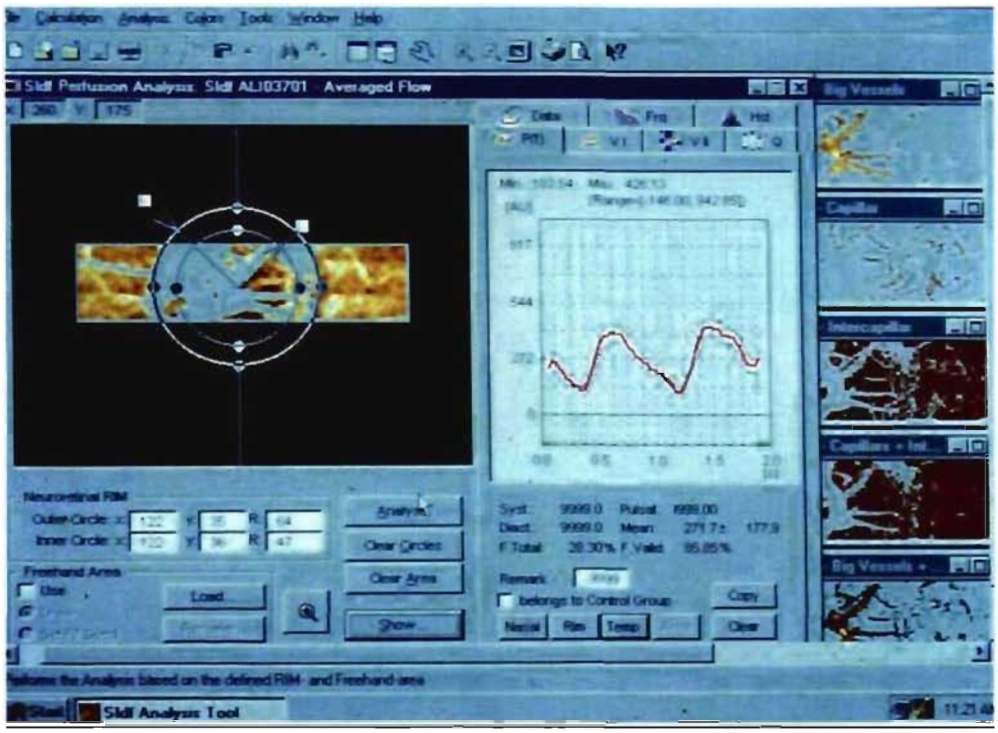
OAG = open angle glaucoma; OHT = ocular hypertension; IOP Pre = intraocular pressure prior to reduction (mmHg); IOP Max = maximum recorded intraocular pressure (mmHg); C/D = cup/disc ratio, M.D. = mean defect; Refr. = error of refraction; Perf. Pr. = calculated ocular perfusion pressure; Med. = medical therapy; Las. = argon laser trabeculoplasty; Sur. = surgery (trabeculectomy); IOP Post = intraocular pressure after reduction (mmHg); Mean % Red'n= Mean percentage reduction of IOP; Durat. (wks) = Mean number of weeks between reduction of IOP and second session of SLDF.

Table 5.5. Mean Rim Z Coordinate Change Relative to Reference Plane (Mean ± SD)

Group	Pre-IOP Red'n (mm)	Post-IOP Red'n (mm)	Change (mm)	P-value
OAG (N=20)	0.104 ±0.09	0.111 ±0.06	+0.007	0.60
OHT (N=20)	0.064 ±0.12	0.062 ±0.12	-0.002	0.85

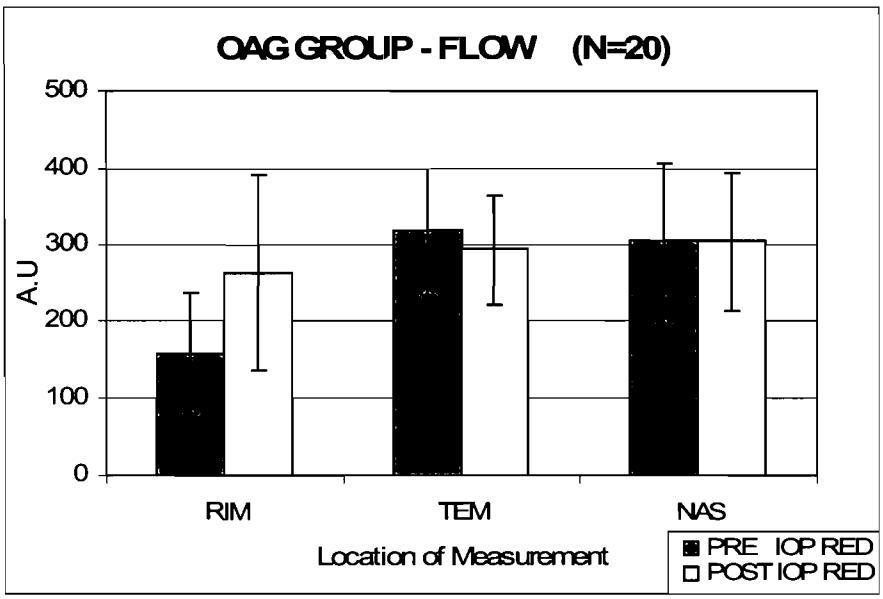
OAG = open angle glaucoma; OHT = ocular hypertension; Pre-IOP Red'n = mean rim Z coordinate before IOP reduction; Post-IOP Red'n = mean rim Z coordinate after IOP reduction.

Figure 5.1



Flow image of temporal peripapillary retinal area using the SLDF full-field perfusion analysis showing the outline of the neuroretinal rim area (left) and a graphic presentation of the heart beat associated pulsation of capillary blood flow (right).

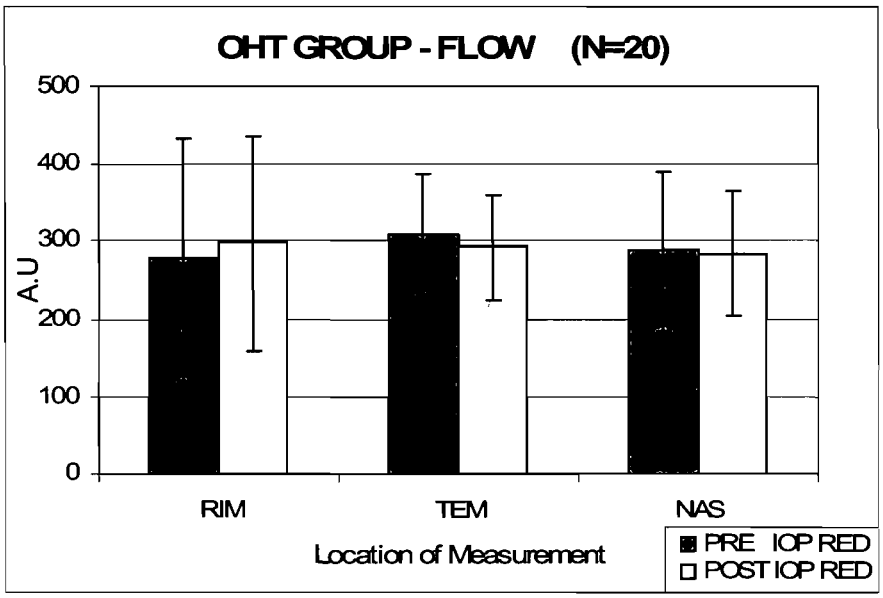
Figure 5.2



SLDF measurements for the parameter flow in the OAG group before and after therapeutic IOP reduction. (*) p=0.001, two-tailed distribution paired t-test.

RIM= neuroretinal rim; TEM= temporal peripapillary retina; NAS= nasal peripapillary retina; PRE IOP RED'N= blood flow before reduction of IOP; POST IOP RED'N= blood flow after reduction of IOP; A.U.= arbitrary units.

Figure 5.3



SLDF measurements for the parameter flow in the OHT group before and after therapeutic IOP reduction. Two-tailed distribution paired t-test.

RIM= neuroretinal rim; TEM= temporal peripapillary retina; NAS= nasal peripapillary retina; PRE IOP RED'N= blood flow before reduction of IOP; POST IOP RED'N= blood flow after reduction of IOP; A.U.= arbitrary units.

DISCUSSION

The introduction of SLDF during the last few years has greatly improved our ability to noninvasively assess the hemodynamics of the optic nerve in glaucoma patients. Studies with SLDF have determined that blood flow in the ONH and peripapillary retina is diminished in OAG patients compared with normals[36,37] or ocular hypertensives[38] and that this decrease occurs in patterns consistent with glaucomatous damage. However, there are few published reports on the association between ONH perfusion changes and IOP reduction in patients that require a lower IOP according to clinical evaluation.

Our results indicate that for a very similar IOP reduction [37% versus 33%], OAG patients had far greater improvements in ONH blood flow than did OHT patients [67.2% versus 7.51%]. On the other hand peripapillary retinal blood flow, expected to be less affected in glaucoma and serving as an internal control, remained stable in both groups. The difference between the two groups did not appear to be related to the OAG group achieving lower IOPs than the OHT group, nor to the six glaucoma patients achieving single digit IOPs. Nor did the difference between the two groups depend on the use of filtration surgery: far larger increases in rim blood flow were seen in the OAG group than in the OHT group even when OAG patients undergoing filtration surgery were excluded.

Numerous studies have reported measurements of blood flow in the ONH, the retina, the choroid and the retrobulbar vasculature in humans and other species. Multiple

techniques have been used in both humans and animals such as fluorescein angiography,[39-44] color Doppler imaging,[45-52] pulsatile ocular blood flow,[53-57] laser Doppler flowmetry[59-61] and scanning laser Doppler flowmetry[36-38.] These studies indicate defective ocular perfusion in glaucoma patients when compared to ocular hypertensives and normal subjects. We have recently reported no statistical difference in the mean SLDF perfusion values between OHT patients and normal subjects (Bizzarro et al, Invest Ophthalmol Vis Sci 2001: 42; S21). Our current results report that compared to OHT patients, OAG patients initially demonstrated significantly lower perfusion values in the neuroretinal rim but not in the peripapillary retina.

Investigators have also reported improved ocular perfusion following IOP reduction in glaucoma patients. Color Doppler imaging demonstrated significant improvements in retrobulbar hemodynamics following trabeculectomy in patients with chronic glaucoma.[48] Pulsatile ocular blood flow measurements similarly demonstrated a significant increase (29%) in ocular blood flow following reduction of IOP by trabeculectomy.[57] In contrast, a recent study using laser speckle flowgraphy showed little change in the superficial ONH circulation following trabeculectomy in a Japanese population of glaucoma patients.[58]

ONH and retinal perfusion have also been evaluated in OAG and OHT patients following use of topical antiglaucoma therapy. The ability of such medications to alter ocular perfusion has been reported by different authors, using different methods to

assess ocular blood flow.[62-71] We observed the largest increase in ONH blood flow in our OAG patients receiving medical therapy, but there were too few patients receiving any particular class of drug to look for the impact of individual drugs on ocular blood flow. However, the observed increase in ONH blood flow in the OAG group could not be attributed to medical therapy *per se* because increased perfusion was observed regardless of whether medical, laser or surgical therapy was used.

Blood flow responses to an induced change in IOP using a suction cup have also been studied in animal models[6,72] and in man[73-75] using fluorescein angiography,[76,77] color Doppler imaging,[47,78] and SLDF.[79] Suction-induced IOP elevations reduced retrobulbar, retinal and ONH perfusion parameters in normal and glaucomatous eyes. Such hemodynamic changes were reversed following normalization of the IOP.

What makes the present findings unique is that we believe they are the first quantitative measurements of perfusion obtained directly from the neuroretinal rim tissue of OAG and OHT patients before and after therapeutic IOP reduction. The inclusion of an OHT comparison group, the members of which had not yet manifested full-fledged glaucoma, is also unique.

Our study design and analysis included numerous controls that permit an increased confidence in the results:

1. The immediately adjacent peripapillary tissue shows almost no change in perfusion measurements. This fact excludes the possibility that media opacities or

ocular optical changes related to pressure reduction, surgery, or the passage of time contributed artifactually to the increases observed, since such increases would have been observed in both tissues. Changes in Doppler values seen in the OAG group are not seen in the OHT group despite almost identical percentage reductions in IOP, suggesting a physiological difference between the two groups.

2. Changes in focus might artifactually change SLDF measurements. In recent studies, which we have confirmed in our laboratory (data not shown), (Lundmark et al, Invest Ophthalmol Vis Sci 37: S265, 1996 and Segawa et al, Invest Ophthalmol Vis Sci 38: S774, 1997), it was shown that as the focal plane is moved either anterior or posterior to the surface of rim tissue, measured values artifactually increase. We have therefore meticulously established the optimal focal plane during each photography session using the tomography capability of the combined flowmeter/tomograph.

3. We also retrospectively evaluated the position of the surface of the neuroretinal rim with respect to the dominant focal plane, the surface of the peripapillary retina. This analysis demonstrates that following IOP reduction, only minimal displacements of several micrometers were observed in each group. These shifts in the mean rim Z coordinate with respect to the focal plane are too small to have artifactually contributed to the observed changes in perfusion values.

4. When patients were matched between the two groups for their IOP following therapy, an improvement in rim blood flow was observed only in the OAG group. As well, within the OAG group, patients with single-digit post-reduction IOPs had

the same magnitude increases in rim blood flow as those patients achieving higher pressures.

5. Incisional surgery was performed on six OAG patients but none of the OHT patients. Among the fourteen OAG patients undergoing medical or laser therapy, a mean 63.2% (p=0.012) improvement of rim flow was recorded, suggesting that the effect observed for the entire group was not related to incisional surgery per se.
6. When patients were matched between the two groups for age, the OAG subgroup maintained the same significant increase in mean rim flow while there was no change in mean rim flow in the OHT subgroup.

The observed changes provide compelling evidence consistent with the hypothesis concerning defective autoregulation of the ONH blood flow in glaucoma. In the OHT group, mean rim blood flow did not change significantly in response to a 33% reduction in IOP suggesting that these patients have intact ONH autoregulation. In contrast, the OAG group demonstrated an increase in the mean rim blood flow of 67% (P = 0.001) in response to a 37% IOP reduction, suggesting that these patients have defective ONH autoregulation. Peripapillary retinal blood flow changes were not significant in OHT as well as OAG patients suggesting an intact retinal autoregulation in both groups.

Since a small subset of OHT patients eventually develop glaucoma, and based on the assumption that a vascular disturbance contributes to the development of glaucomatous

optic neuropathy, it might be expected that some of our OHT patients would show significant improvements in neuroretinal rim blood flow following therapeutic IOP reduction. In fact 4 of the 20 OHT patients (20%) showed an improvement in rim blood flow exceeding 25% following IOP reduction. Thus, a small subset of the OHT patients may be demonstrating defective autoregulation, although as a group their blood flow showed no significant change.

Although the superficial ONH and the peripapillary retina are considered to be both perfused by the central retinal artery, the autoregulation of this flow at the level of the microvasculature may differ between both tissues. This would not be surprising given that the neuroretinal rim is highly abnormal in glaucoma while the peripapillary retina, below the level of the nerve fiber layer, is often (but not always) well preserved. As well, while it is assumed that SLDF does not penetrate beyond 300μm,[20,21] this assumption has not been rigorously verified. Finally, the superficial neuroretinal rim may be receiving a significant contribution to its perfusion by deeper vasculature whose autoregulatory control differs from that of the retina.

The data also indicates that improvement of ONH perfusion in OAG patients may be part of the beneficial response to ocular hypotensive therapy, whether medical, laser or surgery. This concept has been supported by other studies.[48,57]

Further research into the role of ONH blood flow in the different forms of glaucoma, the impact of autoregulation, and the effect of diverse therapies on ocular blood flow should lead to improved understanding of the disease and better therapy for glaucoma patients.

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Chapter 6

Correlation between Finger Blood Flow and Changes in Optic Nerve Head Blood Flow following Therapeutic Intraocular Pressure Reduction

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ABSTRACT

Purpose: To correlate finger blood flow and changes in optic nerve head (ONH) blood flow following therapeutic intraocular pressure (IOP) reduction in open angle glaucoma (OAG) and ocular hypertension (OHT)

Methods: Seventeen OAG and nineteen OHT patients underwent therapeutic IOP reduction followed by a minimum of 4 weeks follow-up. Optic nerve head blood flow measurements were obtained by scanning laser Doppler flowmetry using full-field perfusion image analysis. Finger blood flow was measured using the Transonic laser Doppler Flowmeter. Finger blood flow was measured at baseline, after immersion in warm water (40°C) for 2 minutes (Flow Max), and after immersion in cold water (4°C) for 10 seconds (Flow Min). Patients were identified as vasospastic if their Flow Max/Flow Min > 7. Statistical comparisons were performed using two-tailed distribution paired T-test and Pearson's correlation factor.

Results: For similar mean percentage IOP reduction, vasospastic patients had greater improvements in rim blood flow than did non-vasospastic patients [+35% versus +13%](P=0.01). While there was no difference in rim blood flow changes in the vasospastic versus the non-vasospastic OAG group, the vasospastic OHT group showed 18% increase in rim blood flow while the non-vasospastic OHT group showed 8% decrease. A significant negative correlation was also found in the OAG group between rim blood flow change and Flow Max (-0.681, P=0.003). In contrast, no such correlation was found in the OHT group (+0.144, P=0.556).

Conclusion: OAG patients had a significant negative correlation between changes in rim blood flow and maximum finger Doppler flow. Among OHT patients, increased rim blood flow was only found in the vasospastic group, though this increase was not statistically significant. These results suggest that OAG and OHT patients with the most severe vasospastic disease may show the greatest improvements in rim blood flow following sustained IOP reduction.

Key Words

Glaucoma – Optic Nerve Head Blood Flow – Vasospasm – Autoregulation

INTRODUCTION

The mechanism of damage to the optic nerve head (ONH) in open angle glaucoma (OAG) is almost certainly multifactorial (1). Elevated intraocular pressure (IOP) remains the risk factor most commonly associated with glaucomatous optic neuropathy. However, numerous other variables involved in the development and progression of OAG have been identified (2-7). Vascular risk factors in particular have been extensively studied (8,9). These include systemic blood pressure alterations (10-12), diabetes (13,14), reduced ocular blood flow (15-18) and vasospasm (19-24). Although such vascular risk factors have been postulated several decades ago, only recent technical advances have enabled research into the associated microcirculatory anomalies and their impact on blood flow autoregulation.

Vasospasm is reported as an inappropriate constriction of the smooth muscles of the microcirculation with no recognizable anatomical alterations (25). It can involve different organs simultaneously or successively. Vasospasm is an important factor in the pathogenesis of several diseases such as migraine, Raynaud's syndrome, variant angina and normal tension glaucoma. It can occur in healthy subjects in response to diverse stimuli including exposure to cold, nicotine or emotional stress as well as in association with a variety of diseases, including autoimmune and infectious diseases (26). Although such vasospasm normally leads to reversible functional damage, it may rarely lead to irreversible ischemic changes (27).

Phelps and Corbett in 1985 (28) were the first to suggest the possible role of vasospastic phenomena in the development and progression of glaucomatous optic neuropathy. They found that 47% of their patients with normal tension glaucoma also suffered from migraine. They reported that such frequent occurrence of migraine, Raynaud's syndrome and variant angina suggests generalized vasospastic phenomena. Gasser et al in 1987 (19) described ocular vasospasm in which patients with unexplained scotomas had abnormal capillaroscopic response to cold in the nailfold of the fingers. The scotomas were aggravated by the immersion of a hand in cold water and improved after administration of calcium channel blockers (29). Gasser et al assumed that patients with tendency to vasospasm exhibit ocular vascular reactions similar to those that occur in the capillaries of the fingers. In 1988, Guthauser et al (22) demonstrated a statistically significant relationship between patient's history of cold hands and the outcome of both the visual field cold water test and the nailfold capillaroscopic test. The visual field results were also found to correlate significantly with the capillaroscopic results. In 1988, Drance et al (24), using Doppler blood-flow measurements in the finger and a cold test, showed that in non-glaucomatous subjects, 26% without migraine had a positive vasospastic response while 64% with classic migraine showed such a response. Of the patients with low-tension glaucoma, 65% showed a positive vasospastic response. The Collaborative Normal Tension Glaucoma Study (30) demonstrated a statistically significant increased risk of progression in glaucoma patients suffering from migraine. The relationship between vasospastic changes and structural ischemic changes in glaucoma is still not well understood, though it has been repeatedly

suggested in the literature that insufficient vasospastic regulation in the ONH could explain the abnormal or defective autoregulation of ONH blood flow (31,32).

In a recent study, we have demonstrated a significant improvement (67%, P=0.001) in ONH blood flow in OAG patients following therapeutic IOP reduction whereas patients with ocular hypertension (OHT) did not demonstrate such a change. We attributed the observed changes to defective autoregulation of the ONH blood flow in glaucoma (33). In the present study we extend our findings by examining whether vasospastic OAG and OHT patients demonstrate different ONH blood flow changes in response to therapeutic reduction of IOP when compared to non-vasospastic patients. We also examine the correlation between finger blood flow and changes in ONH blood flow following therapeutic IOP reduction in OAG and OHT.

PATIENTS AND METHODS

Seventeen patients with OAG and nineteen patients with OHT with clinical indication for therapeutic IOP reduction were included in this study. These patients were a subset of a previous study population (33). All patients had a minimum of 20% IOP reduction following medical, laser or surgical intervention as well as a minimum of 4 weeks follow up.

Patients with OAG had glaucomatous optic neuropathy, characteristic nerve fiber bundle visual field defects and gonioscopically open angles with no restrictions for IOP. Ocular hypertensives had a history of repeated IOPs greater than 24 mmHg with normal visual fields and normal or suspect ONH appearance. A detailed medical and ophthalmic history was obtained from all patients, including a questionnaire addressing complaints of cold hands and feet and migraine as well as cardiac problems, intermittent claudication, smoking, alcohol and caffeine intake and exposure to stress.

ONH blood flow measurements were obtained by scanning laser Doppler flowmetry (SLDF) using Heidelberg Retina Flowmeter images (HRF, Heidelberg Engineering, Heidelberg, Germany). The SLDF is a noninvasive instrument combining both a laser Doppler flowmeter with a scanning laser technique. Technical details related to the instrument are discussed elsewhere (34). SLDF measures the amount of backscattered light from the ONH and peripapillary retina. A two dimensional map of microvascular perfusion of the area to be studied (2.7mm x 0.7mm) is thus generated.

SLDF imaging of the ONH was performed at baseline then at a minimum of one month following sustained IOP reduction as previously described (33). Automatic full-field perfusion image analysis (35) was then performed on each of the HRF images and mean values for flow in arbitrary units (au) were obtained from five perfusion images. Our method of SLDF imaging for ONH perfusion as well as its intrasession and intersession reproducibility values has been described in detail in previous publications (36).

Finger blood flow measurements were obtained by the Transonic laser Doppler Flowmeter (Transonic Systems Inc., Ithaca, NY). This device uses a low intensity laser beam transmitted through a fiber optic cable to illuminate the nail-fold capillaries in the finger. A receiver detects light reflected by stationary structures (such as tissue) and moving particles (mainly red blood cells). The latter portion of reflected light undergoes a Doppler frequency shift allowing computation of the proportion of the flow due to red blood cells. Baseline flow was measured on the underside of the end of the middle finger of a randomly selected hand. After a stable flow reading was obtained the hand was immersed in warm water (40°C) for 2 minutes (Fmax). The hand was then immersed in ice-cold water (4°C) for 10 seconds (Fmin) and then finally placed at room temperature for 10 minutes (recovery period) (Figure 6.1). Finger flow measurements were made continuously by the laser Doppler flowmeter and transmitted in real-time to a computer via an interface. Blood pressure measurements were taken when recording flow at baseline, exposure to warm and recovery. Patients were classified into vasospastic and non-vasospastic groups. A vasospastic response was taken as present

when the vasospasticity index (the ratio of maximum flow to minimum flow) exceeded 7, i.e. (Fmax / Fmin > 7). The methodology used for finger blood flow measurements as well as the definition of a positive vasospastic response were both based on a leading study by Drance et al (24).

Changes in ONH and peripapillary retinal blood flow were evaluated using two-tailed distribution paired t-test while group differences were evaluated using one-way analysis of variance (ANOVA). Correlation between changes in ocular blood flow and finger blood flow were performed on each of the OAG and OHT groups using Pearson linear correlation factor. Statistical significance was set at $P < 0.05$.

RESULTS

Data for both ocular and finger blood flow was obtained from 36 patients, 17 OAG patients and 19 OHT patients. Based on their vasospastic response (ratio of maximum flow to minimum flow exceeding 7, i.e. Fmax / Fmin > 7), patients were classified into vasospastic and non-vasospastic groups. There were 22 patients in the vasospastic group (10 patients with OAG and 12 patients with OHT) and 14 non-vasospastic groups (7 patients with OAG and 7 patients with OHT).

Patient characteristics in the vasospastic and non-vasospastic groups are shown in Table 6.1. The mean age (±SD) was 58.7 ± 12.5 years for the vasospastic group (range 42-70 years) and 67.7 ± 8.4 years for the non-vasospastic group (range 52-79 years), [P=0.01, Student's t-test].

The baseline rim blood flow and temporal and nasal peripapillary retinal blood flow were analyzed with a two-way ANOVA. For rim blood flow, the interaction between vasospastic (or not) and diagnosis (OHT and OAG) was not significant [F(1,32)=3.446, P=0.073]. Baseline rim blood flow in vasospastic patients was 187.7± 96.8 au while in non-vasospastic patients it was 281.7±181.7 au [F(1,32)=6.336, P=0.017]. Baseline rim blood flow in OAG patients was 153.9±83.0 au while in OHT patients it was 287.2±154.9 [F(1,32)=15.593, P<0.001]. This analysis indicates that independent of diagnosis there was a significant difference between baseline rim flow values in vasospastic versus non-vasospastic patients. For baseline temporal and nasal

peripapillary retinal blood flow there were no significant interactions or mean differences between groups (all P values are greater than 0.5).

Following sustained therapeutic IOP reduction of 32%, the vasospastic group (Table 6.2) showed a significant improvement in mean rim blood flow of 34.5% [P= 0.01] while mean peripapillary retinal flow showed no significant change [P≥0.18] (two-tailed paired t-test). In contrast, in the non-vasospastic group and following sustained therapeutic IOP reduction of 37% (Table 6.2), mean rim blood flow increased by 13.3% [P=0.32] while mean peripapillary retinal flow showed no significant change [P≥0.27](two-tailed paired t-test).

Among the OAG patients, and following a similar % IOP reduction of 36%, vasospastic patients showed an increase in mean rim blood flow of 64.8% [P=0.003] whereas non-vasospastic patients showed an increase of 62.3% [P=0.09](two-tailed paired t-test) (Table 3). On the other hand, among the OHT patients, vasospastic patients showed an increase in mean rim blood flow of 18.4% [P=0.27] whereas non-vasospastic patients showed a decrease of 8.5% [P=0.38](two-tailed paired t-test) following % IOP reduction of 29% versus 38%, (Table 6.2).

Correlations between the significant changes in ONH blood flow and finger blood flow parameters were then performed on each of the OAG and OHT groups. A significant negative correlation was demonstrated in OAG patients between changes in rim blood

flow following sustained therapeutic IOP reduction and Flow Max (maximum finger flow when the hand was immersed in warm water) [R=-0.681, P=0.003, Pearson linear correlation factor] (Figure 6.2, top). In contrast, no such correlation was found in OHT patients [R=+0.144, P=0.556, Pearson linear correlation factor] (Figure 6.2, bottom). Following therapeutic IOP reduction, OAG patients with low Flow Max demonstrated an increase in neuroretinal rim blood flow of 158 au versus 45 au for the high Flow Max group [P=0.020, Student's t-test]. No correlation was shown between the other peripheral vascular parameters (Flow Base, Flow Min, vasospasticity index, or duration until recovery) and neuroretinal rim flow changes in either OAG or OHT patients.

We then analyzed the detailed medical and ophthalmic history obtained from OAG patients as well the data from the questionnaire addressing complaints of cold hands and feet and migraine as well as cardiac problems, intermittent claudication, smoking, alcohol and caffeine intake and exposure to stress in an attempt to identify the characteristics of OAG patients with low peripheral maximum Doppler flow (Flow Max) as defined by the finger laser Doppler flowmeter results. OAG patients with low "Flow Max" compared to those with high "Flow Max" tended to be somewhat younger in age (63 versus 69 years), showed no difference in the frequency of migraine symptoms, cold hands and feet, smoking or hypertension, and had a similar maximum-recorded IOP (27.5 versus 27.0 mmHg). However, they tended to be more vasospastic (vasospasticity index of 12 versus 6 [P=0.16, Student's t-test]) and reported a higher incidence of current or previous emotional stress (7/8 versus 5/9 patients). We

concluded that patients with low "Flow Max" tended to have predominantly vasospastic profiles rather than atherosclerotic ones.

Table 6.1: Patient Characteristics (Mean ± SD) in Vasospastic Group and Non-vasospastic Group.

	Age Yrs	Sex M/F	IOP Pre	IOP max	C/D Ratio	M. D	Refr. Error	OPP	IOP Post	% Red	Dur. Wks
Vaso-spastic Group N=22	59 ± 13	9/13	25 ± 5	33 ± 5	0.6 ± 0.3	-4.6 ± 7.6	-0.8 ± 3.3	44 ± 8	17 ± 4	32 ± 12	14 ± 5
Non Vaso spastic Group N=14	68 ± 8	8/6	27 ± 6	31 ± 5	0.6 ± 0.3	-4.4 ± 7.6	+0.6 ± 1.9	40 ± 9	17 ± 5	37 ± 14	17 ± 7
T Test	0.01		0.35	0.26	0.70	0.94	0.12	0.14	0.97	0.33	0.24

IOP Pre: intraocular pressure prior to reduction (mmHg); IOP Max: maximum recorded intraocular pressure (mmHg); C/D: cup/disc ratio, M.D.: mean defect of visual field; Refr. Error: error of refraction; OPP: calculated ocular perfusion pressure; IOP Post: intraocular pressure after reduction (mmHg); % Red.: mean percentage reduction of IOP; Dur. (wks): Mean number of weeks between reduction of IOP and second session of SLDF. Two-tailed Student's t-test.

Table 6.2: Changes in neuroretinal rim blood flow in Vasospastic versus Nonvasospastic OAG and OHT patients.

Subjects	VASOSPASTIC GROUP N=22				NONVASOSPASTIC GROUP N=14			
	Pre IOP Red	Post IOP Red	% Change	P value	Pre IOP Red	Post IOP Red	% Change	P Value
OAG + OHT	187.2 ± 96.0	251.7 ± 102.4	+35	0.01	274.7 ± 181.7	311.2 ± 160.3	+13	0.32
OAG	142.3 ± 99.3	234.4 ± 106.5	+65	0.003	169.0 ± 49.8	274.3 ± 137.1	+62	0.09
OHT	224.6 ± 78.6	266.0 ± 101.2	+18	0.27	380.5 ± 207.3	348.0 ± 183.7	-8.5	0.38

OAG: open angle glaucoma; HT: ocular hypertension; Pre IOP Red: flow values before IOP reduction; Post IOP Red: flow values after sustained IOP reduction; % Change: percentage of change in flow.

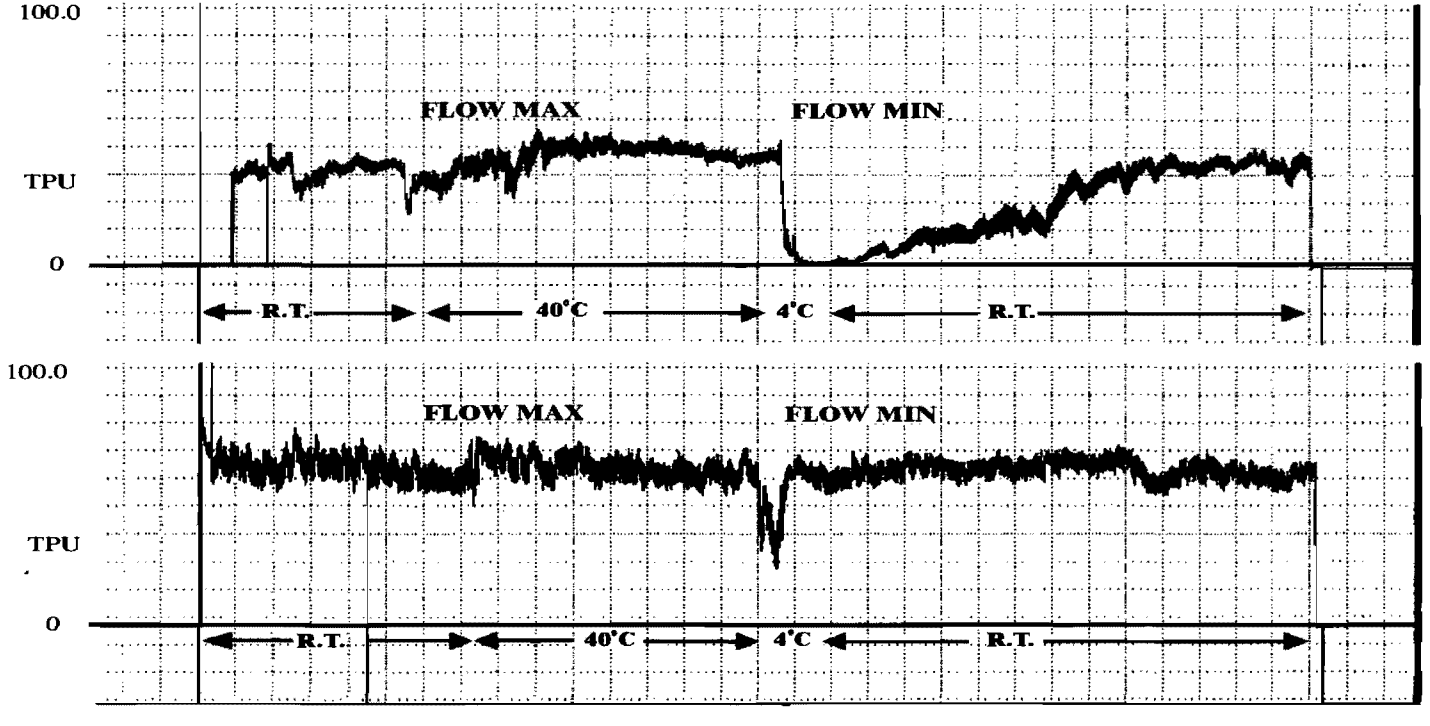
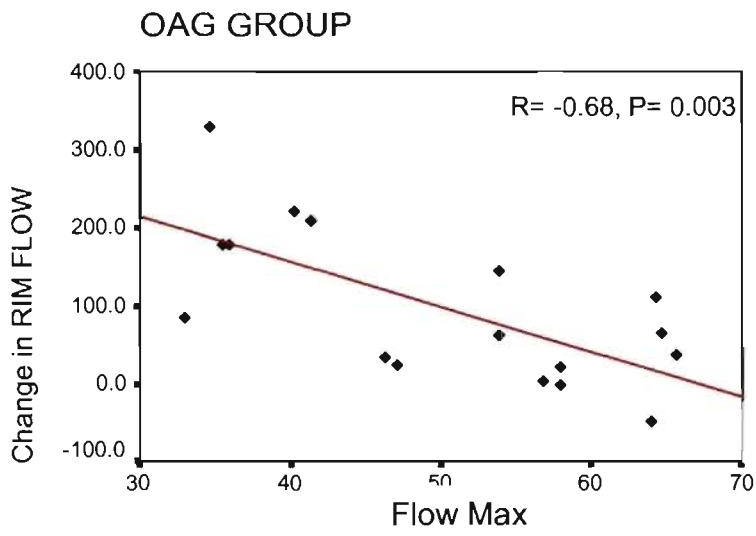
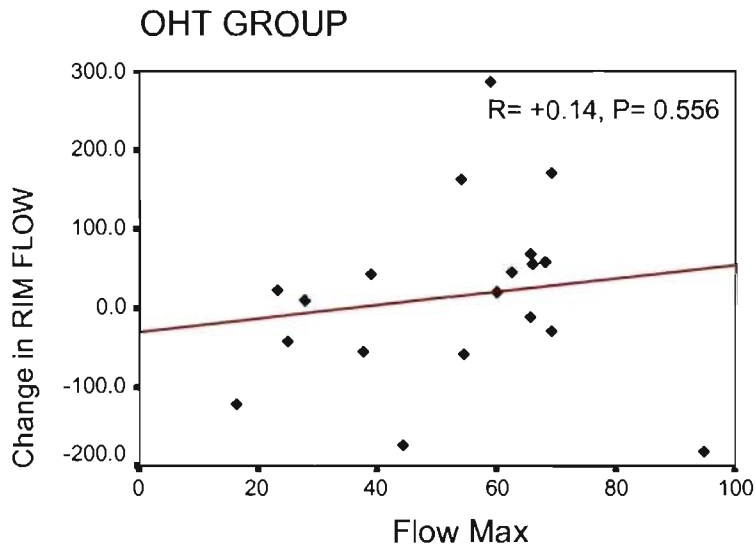


Figure 6.1: Finger Doppler of Vasospastic (Top) and Non-vasospastic (Bottom) Patients. TPU: tissue perfusion units. R.T.: room temperature. The protocol is described in the *Methods*.

Figure 6.2: Correlation between Flow Max (maximum finger blood flow) and Change in Neuroretinal Rim Blood Flow in OAG and OHT patients.





DISCUSSION

This study demonstrates that for a similar percentage of therapeutic IOP reduction, [32% versus 37%] vasospastic patients show greater improvements in ONH blood flow than do non-vasospastic patients [+35% versus +13%]. In ocular hypertensives, improved ONH blood flow following IOP reduction was shown in vasospastic patients [+18%] but not in nonvasospastic patients [-8%]. Changes in ONH blood flow showed a significant negative correlation with maximum finger blood flow in OAG patients but not in OHT patients, such that glaucoma patients with lower finger blood flow had greater increases in ONH blood flow.

We believe that the reported changes in ONH blood flow correlated more with vasospastic indices rather than atherosclerotic ones. The medical and ophthalmic history obtained from OAG patients as well as the data derived from the conducted questionnaire indicate that OAG patients with low maximum finger blood flow (Flow Max) tended to be vasospastic rather than to have significant atherosclerotic cardiac or peripheral vascular disease. OAG patients with low Flow Max also reported being under a higher incidence of current or previous emotional stress. Similar findings were reported among vasospastic patients by Flammer and Prunte (37).

Vasospasm is a systemic disorder. Several studies have reported a high prevalence of migraine, Raynaud's disease and variant angina in patients with peripheral vasospasm (38). Migraine was diagnosed in 26% and Raynaud's disease in 24% of patients with

variant angina (39). In 11% of the patients with variant angina, migraine and Raynaud's disease occurred together (39). Furthermore, an association has been reported between variant angina or migraine and vasospasm in the retinal circulation (40).

Vasospastic patients had significantly lower baseline rim blood flow compared to non-vasospastic patients. We believe that such a difference in baseline ONH blood flow between study groups is related more to shared digital and ocular vasospastic properties than to population differences or instrument variability. Apart from age, which was significantly lower in patients with vasospasm and which reflects a decrease of vasospastic tendencies with increasing age, the two study groups show similar and in some cases identical demographic characteristics. Instrument or technique variability has also been comprehensively evaluated in a prior study of the reproducibility of the SLDF technique (36). In that study we use the automated technique of SLDF full-field perfusion analysis (35) together with obtaining mean values from five high-quality perfusion images. We reported an intrasession variation of 16.0% and an intersession variation of 15.1% in the neuroretinal rim of glaucoma patients.

To the best of our knowledge this is the first report linking peripheral vasospastic disease to changes in ONH blood flow. Previous studies demonstrated that glaucoma patients have significantly increased incidence of peripheral vasospasm (41,42). Other studies observed that visual fields in some glaucoma patients improve following carbon dioxide inhalation and point to possible involvement of an abnormal vascular regulation

(43). Correlations of ONH appearance with age in vasospastic patients suggest that a functional vasospasm might play an important role in the development of glaucomatous optic neuropathy (44). In our study, the mean age of patients with vasospasm was significantly lower than patients without vasospasm (P=0.01). A similar finding was reported by Guthauser et al (22), consistent with the possibility that vasospastic tendencies decrease with age.

Schulzer et al (45) reported that OAG patients could be divided into two groups: patients that were predominantly vasospastic and patients that had predominantly small vessel disease. In the vasospastic group there was a high positive correlation between the amount of visual field damage and the highest intraocular pressure. The authors suggested that this group might have revised manuscript "pressure-dependant" glaucoma. Among the OAG patients with small vessel disease, no such correlation was found and the authors suggested that this group might have "pressure-independent" glaucoma. The present study suggests that ONH blood flow is more sensitive to intraocular pressure in OAG and OHT patients that are vasospastic. It is interesting to speculate that this relationship might underlie the findings of Shulzer et al. Similarly, the Collaborative Normal-Tension Glaucoma Study (46) found that while women with migraines benefited from IOP lowering, patients with a history of cardiovascular disease or a family history of stroke did not, once again linking vasospasticity, IOP, and progressive visual field loss.

Vasospasm has been observed in the posterior ciliary arteries as well as in the choroidal vessels (47,) and was assumed to negatively influence the circulation in the ONH. It is also possible that such vasospasm may occur directly in the vasculature of the ONH. In a recent study by Hasler and coworkers (31) the authors, using single-point laser Doppler flowmetry, report a significant positive correlation between calculated ocular perfusion pressure and blood flow in the choroid of vasospastic subjects. A similar study by Gherghel et al (32), using color Doppler imaging, reported a significant positive correlation between calculated ocular perfusion pressure and both peak-systolic velocity and end-diastolic velocity as well as a significant negative correlation with resistivity index in the central retinal artery of vasospastic subjects. Such correlations did not occur in the control group. Both studies conclude that blood flow-regulating mechanisms might be different between vasospastic and nonvasospastic subjects and point to an abnormal vascular regulation in the retro-ocular circulation of vasospastic patients.

The observed changes in optic nerve blood flow following IOP reduction indicate defective optic nerve vascular autoregulation in much of the studied cohort (33). Defective autoregulation of ONH blood flow has been reported to occur as result of decreased perfusion pressure, increased blood viscosity or increased local resistance (48). Increased local resistance is manifested as a reduced vascular diameter and may be produced by mechanical obstruction by thrombosis, embolization, arteriosclerosis or external compression. Reduced vascular diameter can also be due to a reversible spasm

of the smooth muscle cells in the vessel wall. In their analysis of optic nerve blood flow abnormalities in glaucoma, Flammer and Orgul (48) considered arteriosclerosis as a principal factor for the increased vascular resistance that contributes to defective optic nerve perfusion. Although experimental studies by Hayreh et al (49) indicate that arteriosclerosis might increase the sensitivity to IOP elevations and although some arteriosclerotic patients were shown to present with a sclerotic type of glaucoma (50), Flammer and Orgul (48) believed there was currently very little evidence linking arteriosclerosis to glaucomatous optic neuropathy. Flammer hypothesized increased local resistance to blood flow as a risk factor in the development and progression of glaucoma. He attributed this resistance to a functional rather than a structural change, namely to an abnormal or defective autoregulation of blood flow. Autoregulation implies the capacity of an organ to regulate its perfusion so as to maintain a constant sufficient metabolic supply despite the change in ocular perfusion pressure. An abnormal or defective autoregulation could be expressed not only as excessive vasoconstriction but also as lack of appropriate vasodilatation (51,52).

Our results provide evidence consistent with the hypothesis concerning defective autoregulation of the ONH blood flow in glaucoma and its vasospastic origins. OAG patients showed evidence suggesting defective autoregulation of ONH blood flow and demonstrated a significant negative correlation between changes in ONH blood flow and maximum Doppler flow in the finger. Vasospastic OHT patients also showed a

tendency suggestive of defective ONH autoregulation as manifested by an increase, though insignificant, in ONH blood flow following IOP reduction.

Our findings also identify a subgroup of OAG patients with severe vasospastic disease. We believe these patients demonstrate increased local resistance to blood flow as result of constant vasoconstriction or inappropriate vasodilatation and as manifested by their low Flow Max. These patients were also shown to have the largest improvements in neuroretinal rim blood flow following sustained IOP reduction which points to an abnormal or defective autoregulation in ONH blood flow. OHT patients did not demonstrate this correlation between Flow Max and ONH blood flow suggesting that their vasospastic disease, when present, may be less pervasive.

In the OHT group, vasospastic patients showed a similar trend of improved ONH blood flow whereas non-vasospastic patients did not. In a clinical context, a small subset of OHT patients eventually develop glaucoma, and based on the assumption that a vascular disturbance contributes to the development of glaucomatous optic neuropathy, it might be expected that some of our OHT patients would show significant improvements in neuroretinal rim blood flow following therapeutic IOP reduction. In fact 3 of the 19 OHT patients (16%) showed such an improvement in rim blood flow. Thus, a small subset of the OHT patients may be demonstrating defective autoregulation, although as a group their blood flow showed no significant change.

Such a behavior of OHT patients raises the question of whether this factor might identify OHT patients at risk for developing glaucoma.

The relatively small sample size as well as the large variation might have similarly limited our results related to both the OAG and OHT subgroups. However, to the best of our knowledge, we believe the findings of the present study suggest for the first time an abnormal or defective autoregulation occurring in the ONH of vasospastic subjects. It also shows a significant correlation between the presence of lower peripheral blood flow (Flow Max) in OAG patients and improvement in ONH blood flow with therapeutic IOP reduction and thus provides evidence that systemic vasospasm or vascular dysregulation might actually underly such a defective ONH autoregulation in glaucoma.

We believe our data may form the basis for a larger long-term study to examine the relationship between digital and ocular vasospasm and their impact on autoregulation of ONH blood flow and on progression of glaucomatous optic atrophy in OAG and OHT. We believe that such studies will eventually lead to a better understanding of the disease process as well as to novel therapeutic approaches for our patients.

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Chapter 7

Relationship between Central Corneal Thickness and Changes of Optic Nerve Head Topography and Blood Flow following IOP Reduction in Open Angle Glaucoma and Ocular Hypertension

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ABSTRACT

Purpose: To investigate changes in optic nerve head (ONH) topography and blood flow after therapeutic intraocular pressure reduction and to correlate them with central corneal thickness (CCT).

Methods: Sixteen patients with open angle glaucoma (OAG) and 16 patients with ocular hypertension (OHT) underwent Heidelberg Retina Tomography (HRT) and Scanning Laser Doppler Flowmetry (SLDF) in one eye before and at least two months following a mean 35% sustained therapeutic reduction in intraocular pressure IOP. Patients were assigned to a thin" or thick" group based on their median CCT.

Results: Compared with 16 patients with thick corneas (mean±SD central corneal thickness, 587±31 μm), the16 patients with thin corneas (518±32 μm) had greater reductions in mean (36±32 vs 4±36 μm, P=0.003) and in maximum cup depth (73±107 vs 4±89 μm, P=0.02). These changes were not statistically significantly different between the patients with open-angle glaucoma and those with ocular hypertension. Smaller mean±SD improvements in neuroretinal rim blood flow were seen in patients with thinner corneas compared with those with thicker corneas (35±80 vs 110±111 arbitrary units, P=0.04).

Conclusions: OAG and OHT patients with thinner corneas show significantly greater shallowing of the cup, a surrogate marker for lamina cribrosa displacement (compliance) and smaller improvements of neuroretinal rim blood flow following IOP reduction.

INTRODUCTION

Altered lamina cribrosa compliance has long been postulated to have a role in the development of open angle glaucoma (OAG). Lamina cribrosa mobility has been studied in in *ex vivo* human [1-6] and monkey [7,8] eyes, in living human [9-13] and monkey eyes [14-15]and in histological [1,8,16-18]studies. Findings from some suggest that there may be an initial hypercompliance in early glaucoma followed by reduced compliance (i.e. increased rigidity) later in the course of the disease[8,15,19]. In most patients with glaucoma, the central lamina cribrosa is covered by little or no neural or glial tissue. Therefore, lamina cribrosa compliance can be readily estimated using confocal scanning laser tomography by examining the position of the base of the cup relative to the retinal surface following intraocular pressure (IOP) changes. [9,11-13,]

Considerable evidence suggests that abnormal optic nerve blood flow has a role in the development of glaucomatous optic neuropathy [20]. Recent data suggests that optic nerve head (ONH) neuroretinal rim blood flow improves significantly in open angle glaucoma (OAG) patients after sustained therapeutic IOP reduction [21]. Among patients with ocular hypertension (OHT), such improvements were limited to vasospastic subjects [22]. The prognostic significance of these blood flow changes remains to be determined.

Findings suggest that the presence of a thin cornea is linked to the development of glaucoma among patients with OHT [23] as well as to the severity of OHT [24, 25] and OAG. [26, 27] In both OHT and OAG, a thin cornea is more strongly associated with

disease severity than IOP. [23, 27] Underestimated Goldmann tonometric pressures appear to only partly explain the relationship between thin corneas and increased glaucoma risk. The other mechanisms underlying this relationship are unknown. Cornea thickness has been linked to scleral thickness.[28-30] In this study, we examine the relationship between central corneal thickness (CCT) and lamina cribrosa compliance. Because the blood vessels that feed the ONH run through the lamina cribrosa, we also examine changes in neuroretinal rim blood flow that occur with IOP-dependant lamina changes.

METHODS

The study protocol was approved by the research committee of Maisonneuve-Rosemont Hospital and all patients signed an informed consent form.

Patients with OAG had gonioscopically confirmed open angles and manifested at least 2 of the following 3 criteria: characteristic nerve fiber bundle visual field defects, glaucomatous optic neuropathy and a history of IOP greater than 21 mm Hg. Patients with OHT had a history of IOP greater than 24 mm Hg on at least 2 occasions, normal visual fields, and normal or suspect ONH appearance based on slitlamp biomicroscopy. Subjects were excluded if any abnormal ocular findings were present other than pseudophakia, if significant media opacities precluded scanning laser Doppler flowmetry (SLDF) imaging, and if they were unable to comply with the study protocol. Medical and ocular histories were recorded, and IOP, refractive errors, and best corrected visual acuity were measured before the baseline study visit. A basic ophthalmologic examination, including biomicroscopy, ophthalmoscopy, and gonioscopy, was performed, and the visual field was assessed using automated perimetry (Humphrey Field Analyzer, program 24-2; Humphrey Instruments, San Leandro, California).

Thirty-two patients having clinical indication for IOP reduction were recruited from the hospital glaucoma clinics and underwent confocal scanning laser tomography with the Heidelberg Retina Tomograph (HRT, Heidelberg Engineering, Heidelberg Germany, version 2.01) and SLDF of the ONH (using Heidelberg Retina Flowmeter images and

software version 3.3 [31]) before and at least two months following a minimum IOP reduction of 20%. For HRT imaging, the mean topographies were derived from three high quality images. SLDF values for neuroretinal rim blood flow were derived from the mean of five high quality images as described previously. [21]

As indicated clinically, IOP was reduced, using topical hypotensive medications, argon laser trabeculoplasty or filtration surgery. All patients were treated by the same physician (MRL). One eye was studied in each patient. If both eyes required therapy, the eye with the clearer media was chosen. We used the HRT stereometric parameters of mean cup depth and maximum cup depth to estimate lamina cribrosa position in micrometers (μm) before and after IOP reduction. We used the SLDF parameter of flow in all valid pixels overlying the neuroretinal rim to determine neuroretinal rim blood flow in arbitrary flow units (AU) before and after IOP reduction.

Central corneal pachymetry was determined using an ultrasound pachymeter, (DGH 500 Pachette, DGH Technology, Fraser, PA, USA) using the mean of the three closest of five consecutive measurements. Values are presented as mean +/- standard deviation (SD). Statistical evaluations were performed using Pearson's correlation test and Student's T-Test. Statistical significance was set at $P < 0.05$. An analysis of covariants (ANCOVA) was used to control for covariables.

RESULTS

Patient characteristics are shown in Table 7.1. There were 16 OAG and 16 OHT patients. Patients were assigned to the thin group or to the thick group based on the median CCT. In order to keep the groups balanced with respect to diagnosis, the eight OAG patients with thinnest corneas were grouped with the eight OHT patients with thinnest corneas to form the "Thin Group". Clinical parameters other than CCT did not differ significantly between the thin (N=16) and thick (N=16) groups (Table 7.1).

The ONH stereometric parameter of mean cup depth was reduced by a mean value of 36 +/- 32 um in the thin CCT group, but 4 +/- 36 um in the thick CCT group, a difference that was statistically significant (P = 0.03, ANCOVA) (Table 7.2 and Figure 7.1). Maximum cup depth was reduced by 73 +/- 107 um in the thin CCT group but only 4+/- 89 um in the thick CCT group, a difference which statistically significance (P = 0.02, ANCOVA).

The relationship between corneal thickness and shallowing of the cup was present in both the OAG and the OHT groups and was not significantly different between the groups (P = 0.29 and P = 0.18 for mean and maximum cup depths, respectively, ANCOVA) (Table 7.3).

We also looked for significant changes of cup depth in individual eyes. The standard deviation of cup depth for the 3 images performed at each of 2 sessions (before and after IOP reduction) was calculated. Then, the number of eyes in which the cup depth

changed by >4 SDs for that eye was calculated. One eye for which we were unable to locate the original images was excluded from this analysis. For mean cup depth, 8 of 15 eyes showed significant shallowing in the thin CCT group, while 3 of 16 eyes showed significant shallowing in the thick CCT group, a difference that was significant by X^2 analysis (P = 0.04). The same analysis for maximum cup depth yielded 4 of 15 eyes showing at least 4-SD shallowing in the thin CCT group compared with 1 of 16 eyes in the thick CCT group, a difference that was not significant (P = 0.1).

We further confirmed the difference in topographical changes by performing the Mann-Whitney rank order test for changes of mean and maximum cup depth. This test confirmed that, compared with the thick CCT group, the thin CCT group had significantly greater reductions in mean cup depth (P=0.02), while the greater reduction in maximum cup depth in the thin CCT group did not reach statistical significance (P = 0.13).

Smaller improvements in neuroretinal rim blood flow were seen in OAG and OHT patients with thinner corneas compared with those with thicker corneas. This difference was statistically significant in OAG and OHT patients and remained significant after controlling for percentage IOP reduction (P = 0.04, ANCOVA). This difference was significant in the OAG group but not in the OHT group (Table 7.4 and Figure 7.2). We also looked for significant changes of rim flow in individual eyes. The standard deviation of rim flow for the 5 images performed at each of 2 sessions was calculated. Then, the number of eyes in which the rim flow changed by more than 4 SDs for that

eye was calculated. Seven of 16 eyes showed significant increases in rim flow in the thick CCT group, while 2 of 16 eyes showed such increases in the thin CCT group, a difference that was significant by X^2 at P = 0.05. Using a cut-off of 3 SDs gave a more significant X^2 result of P = 0.01 (9 of 16 in the thick CCT group vs 2 of 16 in the thin CCT group).

Table 7.1: Characteristics of combined OAG and OHT patients based on median central corneal thickness (CCT)

	COMBINED OHT + OAG PATIENTS		
	Thin C.C.T N=16	**Thick C.C.T N=16**	**P Value**
Age (Yrs)	64.0±12.8	62.2±12.4	0.69
C.C.T (μm)	518 ± 32	587 ± 31	<0.001
Clinical C/D Ratio	0.58 ± 0.24	0.56 ± 0.26	0.84
Mean Defect of VF (dB)	-3.2 ± 4.1	-4.1 ± 6.9	0.68
IOP before reduction (mmHg)	29±4	29±5	0.82
Maximum IOP (mmHg)	29 ± 4	29 ± 5	0.82
% IOP Reduction	38 ± 13	30 ± 12	0.09

Clin. C/D Ratio: Clinically determined cup/disk ratio: Mean Defect of VF: Mean mean defect on recent Humphrey Automated Perimetry, Program 24-2 SITA standard. Tmax: Maximaum recorded untreated IOP for the eye. % IOP Reduction: Percent reduction of IOP at the time of the second imaging session.

Table 7.2: Change in Topographic Parameters following sustained IOP reduction among patients with OAG and OHT.

	COMBINED OHT + OAG PATIENTS			
Parameter	Thin C.C.T N=16	Thick C.C.T N=16	Unit Difference	P Value
Mean Cup Depth (μm)	-36 ± 32	-4 ± 36	-32	0.003
Max Cup Depth (μm)	-73 ± 107	-4 ± 89	-69	0.020

Analysis of covariance ANCOVA

Table 7.3: Change in Topographic Parameters following sustained IOP reduction among OAG patients and OHT patients.

	OAG Patients			**OHT Patients**		
Parameter	Thin C.C.T N=8	Thick C.C.T N=8	Unit Diff.	Thin C.C.T N=8	Thick C.C.T N=8	Unit Diff.
Mean Cup Depth (μm)	-43 ± 29	-7 ± 41	-36	-29 ± 34	-1 ± 33	-28
Max. Cup Depth (μm)	-95 ± 104	22 ± 88	-73	-50 ± 110	14 ± 92	-64

CCT: central corneal thickness, Diff: difference in units between the two changes

Table 7.4. Changes in Rim Blood Flow following sustained IOP reduction in OAG, OHT and combined OAG+OHT groups.

	Thin CCT	**Thick CCT**	**P-value**
OAG (N=16)	46 ± 82	150 ± 106	0.048
OHT (N=16)	23 ± 83	71 ± 110	0.34
OAG+OHT (N=32)	35 ± 80	110 ± 111	0.037

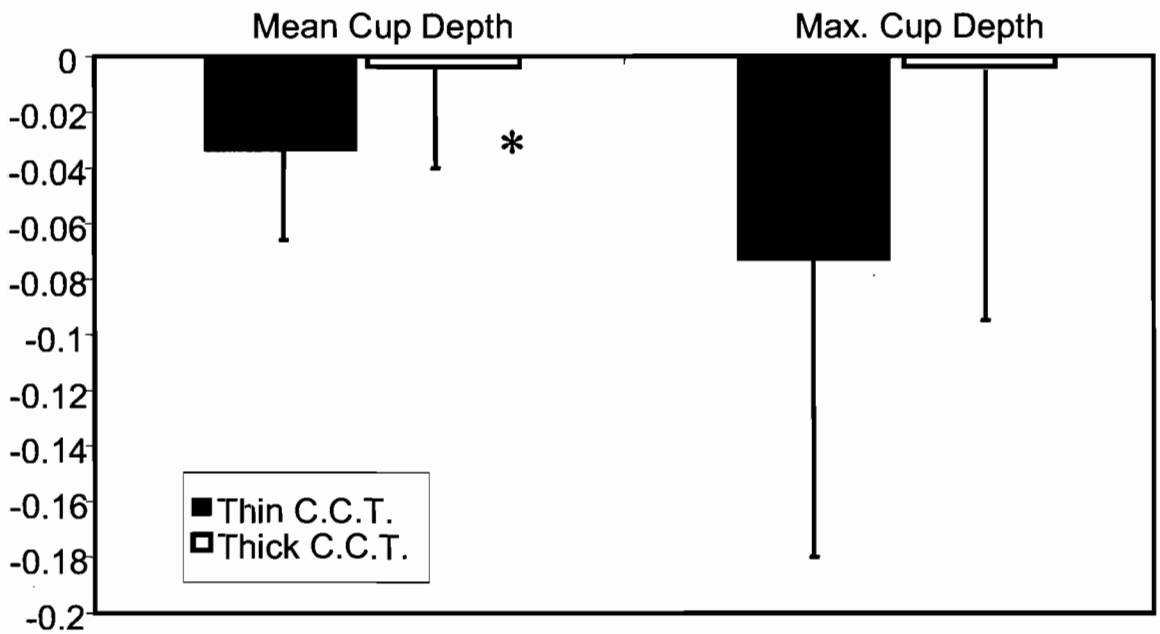

Figure 7.1: Change in Topographic Parameters following IOP reduction for all patients (combined OAG+OHT patients) (N=32).

Measurements are in millimeters. * indicates $P < 0.05$.

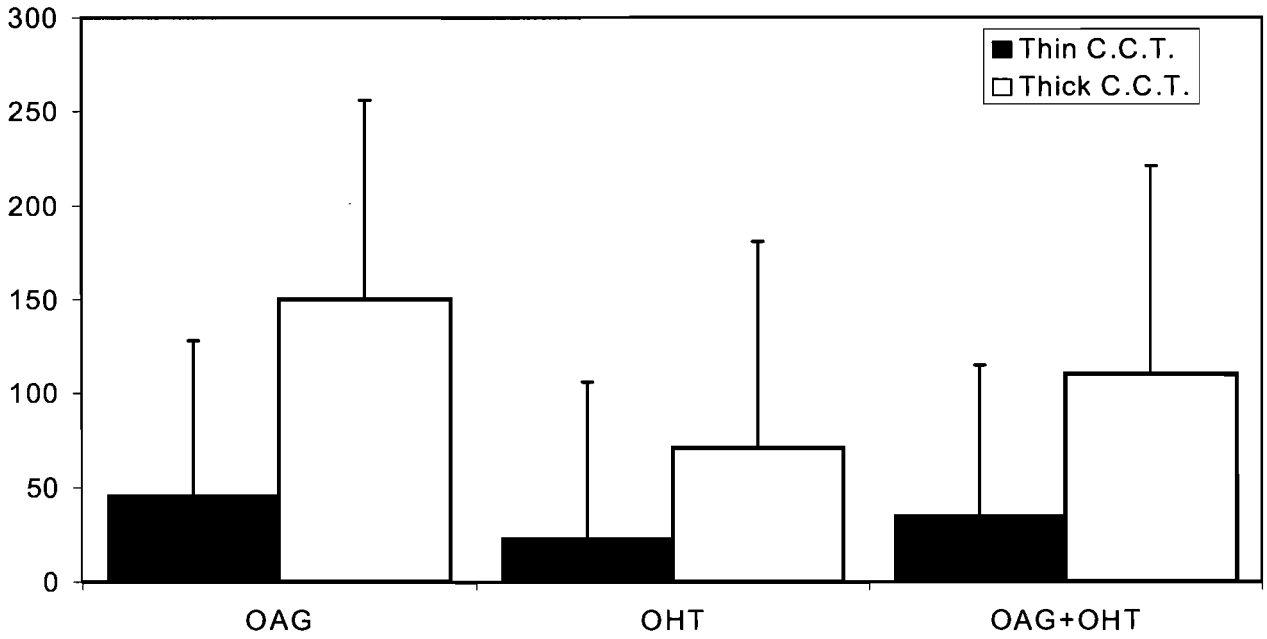

Figure 7.2: Change in Neuroretinal rim blood flow following IOP reduction for combined OAG+OHT patients (N=32) and for OAG and OHT subgroups. Measurements are in arbitrary units. * indicates $P < 0.05$

DISCUSSION

The results of this preliminary study suggest that OHT and OAG patients with thin corneas have greater forward displacement of the base of the cup, a surrogate marker for lamina cribrosa position, upon sustained IOP reduction than do their cohorts with thicker corneas. Patients with thin central corneas also appear to have smaller improvements in neuroretinal rim blood flow following IOP reduction than patients with thicker central corneas.

A thin central cornea is emerging as a major risk factor for severity of OHT and OAG[23-27] . Diurnal and long-term IOP fluctuations are also a major risk factor for progression in OAG.[32-34] These results suggest that a thin cornea may be a marker for physiological differences in the biomechanical properties of the lamina cribrosa. In other words, it may be that a thin cornea is connected to a thin sclera, which, in turn is connected to a thin lamina. Assuming identical material properties, a thin lamina would have greater compliance (less rigidity) than a thick lamina. [6] A thin lamina should then manifest by greater displacements in response to diurnal or long-term IOP fluctuations. Greater lamina displacements would lead to greater damage to axons and to capillaries that pass through it, and therefore to more optic nerve damage. [35]

Larger increases in rim blood flow following IOP reduction were observed in the thick CCT groups. Because patients with thick corneas may have a reduced risk of

progression or of reaching an advanced state of glaucoma, [23-27] this finding suggests that improved blood flow in response to therapy may be a good prognostic sign in glaucoma. In patients with thin corneas, it is conceivable that the vasculature has become more damaged due to repetitive movements of the more compliant lamina. In these patients, the vasculature may be less able to respond to IOP reduction with a beneficial increase in blood flow.

Smaller increases in optic nerve head blood flow may also be present upon IOP reduction because the microvasculature passing through the lamina cribrosa may become compressed by the large forward displacement of the laminar sheets. Laminar sheet compression has been previously described in glaucoma.[16] The data thus suggests an inter-relationship between the mechanical and vascular properties of the optic nerve head.

However, our data on neuroretinal rim blood flow may, in fact, be misleading. In the eyes with more compliant laminas, it is possible that laminar (as opposed to neuroretinal rim) blood flow after IOP reduction was greatly increased. This increase in laminar blood flow (not measured by our method) may have manifested as a less impressive increase in neuroretinal rim blood flow because of shunting. Future research should examine lamina cribrosa blood flow and neuroretinal rim blood flow.

A review of the literature suggests that after an initial hypercompliant phase, the lamina cribrosa becomes more rigid in glaucoma [1-9, 14-15,35,36]. One interpretation of these

findings is that increased laminar rigidity contributes to axonal loss. Another interpretation is that increased laminar rigidity follows axonal loss. Findings from the current study suggest that patients with thick central corneas have a more rigid lamina cribrosa. Because other studies [23-27] have demonstrated a lower risk of progression in patients with thick central corneas, increased laminar rigidity may be a biological response that is protective to axons in this disease. Although the mechanisms of this protection remain unknown, the results of our study suggest that improved blood flow to the neuroretinal rim following IOP reduction may be involved in this protective effect.

Our findings also suggest a potential method for determining the risk level for an individual glaucoma patient. Patients with high lamina cribrosa mobility or poor vascular response to IOP reduction may be at greater risk of progressive disease and may be targeted for more aggressive or alternate therapies. Although the results presented here are preliminary and the mechanistic links speculative, they may serve as a conceptual framework for more detailed future studies.

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Chapter 8

Changes in Optic Nerve Head Topography following Therapeutic Intraocular Pressure Reduction in Glaucoma Patients and Ocular Hypertensives

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ABSTRACT

Purpose: To compare changes in optic nerve head (ONH) topography following therapeutic intraocular pressure (IOP) reduction in open angle glaucoma (OAG) and ocular hypertension (OHT) patients.

Methods: Twenty patients with OAG and twenty patients with OHT with clinical indication for therapeutic IOP reduction were prospectively enrolled. IOP reduction was achieved by medical, laser or surgical therapy. All patients had IOP reductions more than 20% and a minimum of four weeks follow-up. Scanning laser topographic measurements were performed before and after IOP reduction using the Heidelberg Retina Tomograph (HRT) (Heidelberg Engineering, Heidelberg, Germany). A mean topography of three images was obtained and topography differences were computed using software v2.01. Statistical evaluations were performed on both groups using a one-way analysis of variance (ANOVA).

Results: Twenty patients with OAG had IOP reduction of 37% ± 16% (Mean ± SD) and a follow-up duration of 14 ± 6 weeks after treatment. Twenty ocular hypertensives had IOP reduction of 33% ± 12% and a follow-up duration of 14 ± 6 weeks after treatment. Following sustained IOP reduction, none of the changes in topographic parameters showed a statistically significant difference between the two groups ($P \geq 0.29$).

Conclusion: Following a similar percentage of therapeutic IOP reduction, mean change of ONH topographic parameters did not differ significantly between the OAG and OHT groups. This finding suggests that the structural compliance of the ONH tissue was

similar in our population of OAG and OHT. ONH compliance changes may therefore be hypothesized to occur early in the course of glaucomatous disease process.

INTRODUCTION

Cupping of the optic nerve head (ONH) is an important clinical sign in the diagnosis and follow-up of glaucoma. Regression of ONH cupping and increase in neuroretinal rim area has been reported in congenital and infantile glaucoma (1,2) as well as in adults (3-5) following therapeutic intraocular pressure (IOP) reduction.

Structural changes in ONH parameters over time have been demonstrated using a variety of diagnostic techniques including evaluation of stereo disc photos (6-8), photogrammetric methods (9), computer videographic imaging (10,11) and confocal scanning laser ophthalmoscopy (CSLO)(3-5).

CSLO provides quantitative, objective and reproducible topographical measurements of the ONH (12-16). The technique has been reported to differentiate between optic nerve heads of normal individuals and glaucoma patients with sensitivities ranging from 62%-87% and specificities ranging from 73%-94% (12,17,18). Using CSLO, increased rim area and volume, reduced cup area and volume, and reduced mean and maximum cup depth have been observed in response to sustained reduction of intraocular pressure (IOP) in glaucoma patients (3-5). Improvement in topographic parameters were found to correlate with the percent reduction of IOP (3,5).

Improvements in disc parameters were also shown to occur in eyes with damage at relatively early stage but not in eyes with advanced glaucoma. Experimental studies by Shirakashi et al (19) and Coleman et al (20) reported significantly less anterior

displacement of the optic discs with larger and deeper cups whereas Zeimer et al (21) reported a decreased compliance of the ONH as the visual field worsened.

Studies of the mechanical deformation of the ONH are essential to identify the properties of underlying connective tissues and their susceptibility to damage. Deformation of the surface of the ONH relative to the peripapillary retina as well as displacement of the lamina cribrosa after artificial changes of IOP has been recognized and quantified in *ex vivo* human (21-26) and monkey (27,28) eyes, in *living* human (3-5,29,30) and monkey eyes (23,31,32) and in histological studies (22,27, 33-35).

Burgoyne et al (36), using digitized image analysis, reported that changes in ONH compliance might occur early in the course of chronic experimental glaucoma. Yan et al (22) evaluated the position of the lamina cribrosa in enucleated normal human ONH when fixed after chronic exposure to elevated IOP. Elevated IOP caused the lamina to deflect posteriorly without affecting its thickness. The displacement was maximal at the periphery and minimal at the centre of the ONH. Bellezza et al (37) similarly reported a permanent posterior deformation of the lamina cribrosa and anterior scleral canal wall in early experimental glaucoma. Such deformation was attributed to damage to the connective tissue of the ONH. These changes were also evident through 3-D reconstruction of the ONH connective tissues (38).

Other studies reported structural changes in the elastic component of the lamina cribrosa in response to constant fluctuation of IOP. Such structural changes were thought to persist after reduction of IOP permanently changing the mechanical properties of the

tissue (39). Altered elastic strength and stiffening of the mechanical support of the ONH has thus been invoked as a possible contributing factor to the susceptibility of some eyes to further damage contributing to the progression of glaucomatous optic neuropathy (21,26-40).

The purpose of our study was to investigate the mechanical properties of the glaucomatous ONH in a real-life context. We used CSLO to measure morphological changes of the ONH following sustained therapeutic IOP reductions. These changes were compared to those found in a group of ocular hypertension (OHT) patients also undergoing similar sustained therapeutic IOP reduction. The OHT group is considered to have the earliest stage of glaucoma. Therefore by comparing these two groups we hoped to evaluate how ONH compliance changes in very early as opposed to established glaucoma.

PATIENTS AND METHODS

The study was approved by the research committee of Maisonneuve-Rosemont Hospital, University of Montreal. An informed consent was obtained from each patient before enrollment into the study. Twenty patients with OAG and twenty patients with OHT with clinical indication for therapeutic IOP reduction were prospectively recruited from the glaucoma clinics of the hospital. Patients with OAG had gonioscopically open angles and fulfilled at least two of the following three criteria: a history of intraocular pressure (IOP) greater than 21 mmHg, characteristic nerve fiber bundle visual field defects and glaucomatous optic neuropathy. Ocular hypertensives had a history of IOP greater than 24 mmHg on at least two occasions, normal visual fields and normal or suspect optic nerve head appearance based on slit-lamp biomicroscopy.

Imaging of the ONH was obtained using the Heidelberg Retina Tomograph (HRT, Heidelberg Engineering, GmbH, Heidelberg, Germany) (12,15). The HRT employs a scanning ophthalmoscope with an infrared diode laser of wavelength 670 nm and confocal optics for image acquisition. The focal plane of the laser is located in front of a detector, which measures the intensity of the light reflected from the fundus. By varying the focal plane of the laser, cross-sectional images of the ONH can be obtained. The topographic image is a series of 32 optical sections at consecutive focal planes. Each image consists of 256 pixels x 256 pixels, each pixel corresponding to the ONH height at that location. The depth of each topographic image series ranges from 0.5mm to

4.0mm depending on ONH morphology. Total image acquisition time is approximately 1.6 seconds.

For image acquisition, pupils 3 mm in diameter or smaller were dilated. The fundus camera was adjusted until a focused, evenly illuminated and centered view of the ONH was obtained. The patient was asked to use the fellow eye for fixation and to refrain from movement and blinking. Using a 10-degree field and the appropriate focus and depth settings, a total of 5 images were then acquired sequentially. The angulation of the fundus camera as well as its distance from the eye was kept constant throughout the session. All images were reviewed by the same observer and prior to data analysis the best three images in terms of focussing, centration and brightness were chosen. A mean topography image was then created from these three images.

All patients then underwent IOP reduction by medical, laser, or surgical intervention. All patients in the study had sustained IOP reduction of 20% or more, as well as a minimum of four weeks follow-up between the intervention and the second HRT imaging session. For those patients undergoing incisional surgery, the second imaging session was at least 12 weeks following the intervention, in order to avoid questions of hypotony, edema and inflammation. Patients then underwent a second session of topography using the same settings previously used for the previous topography (scan area, focussing, sensitivity). The best three images were similarly chosen and a mean topography was obtained and used for analysis.

The optic disc margin contour line was drawn on the Pre-IOP reduction mean topography image with the aid of stereo photographs of the ONH. The optic disc margin contour line was then exported from the Pre-IOP reduction mean topography image to the Post-IOP reduction mean topography image using the capabilities of the HRT software version 2.01. The parameters investigated in this study were cup area, cup/disc ratio, rim area, mean cup depth, cup volume, rim volume, maximum cup depth, cup shape index and height variation contour. These parameters were measured relative to the standard reference plane. The standard reference plane was defined as 50 microns below the average height of the contour line in an inferior temporal segment between 350 degrees and 356 degrees.

Topographic parameters in both study groups before and after IOP reductions as well as topographic differences in each study group were then compared using two-tailed Student's t-test and analysis of variance (ANOVA) with P values < 0.05 considered to be statistically significant.

RESULTS

Pre and Post-IOP reduction HRT imaging was performed on 22 patients with OAG and 21 patients with OHT. Good quality images were obtained from 20 OAG patients and 20 OHT patients and were analyzed. Causes of poor quality images were mainly lens or vitreous opacities.

Patient characteristics are presented in Tables 8.1 and 8.2 The mean age (± SD) was 66.7 ± 10.9 years for the OAG group (age range 42-79 years) and 57.2 ± 11.6 years for the OHT group (age range 42-75 years). There were 9 males (45%) and 11 females (55%) in each of the two study groups. There was no statistically significant difference between the OAG group and the OHT group in the means of maximum-recorded IOP, refractive error and ocular perfusion pressure. Statistically significant differences were demonstrated between the two groups in the means of age, IOP before reduction, clinical C/D ratio and visual field mean defect [$P \leq 0.011$, two-tailed Student's t-test] (Table 8.1). Statistically significant difference was also demonstrated between the OAG group and the OHT group in the mean IOP after reduction [P=0.0002, two-tailed Student's t-test]. There were no statistically significant differences between the two groups in the percentage of IOP reduction and the follow-up duration [P=0.349 and 0.827 respectively, two-tailed Student's t-test]. The mean percentage of IOP reduction was 37% in the OAG group and 33% in the OHT group (Table 8.2).

Therapeutic IOP reduction was attained by ocular hypotensive drugs in 4 of the 20 OAG patients (20%) and 12 of the 20 OHT patients (60%). Ten OAG patients (50%)

and 8 OHT patients (40%) had therapeutic IOP reduction by argon laser trabeculoplasty while 6 OAG patients (30%) and none of the OHT patients had filtering procedures.

In our study, the measured reference plane height for each of the two study groups did not change significantly between Pre-IOP reduction images and Post-IOP reduction images. Average reference plane height in the OAG group before and after IOP reduction was 0.303 ± 0.087 mm and 0.310 ± 0.072 mm respectively (P=0.79, two-tailed paired t-test). Average reference plane height in the OHT group before and after IOP reduction was 0.326 ± 0.146 mm and 0.324 ± 0.123 mm respectively (P=0.97, two-tailed paired t-test).

Table 8.3 summarizes the baseline HRT topographic parameters (Mean ± SD, unit difference, % difference, P value) for both the OAG group and the OHT group. Prior to IOP reduction, OAG patients demonstrated significant differences in the topographic parameters of cup area (P<0.0001), cup/disc ratio (P<0.0001), rim area (P=0.014), rim volume (P=0.006) and cup shape measure (P=0.001) when compared to OHT patients. Marginally significant differences were demonstrated between OAG and OHT patients in the topographic parameters of cup volume (P=0.067). No statistically significant difference was observed between the two study groups in the topographic parameters of mean cup depth (P=0.617), maximum cup depth (P=0.594) and height in contour (P=0.387) [two-tailed Student's t-test].

Tables 8.4 and 8.5 summarize HRT topographic parameters before and after therapeutic IOP reduction (Mean ± SD, unit change, % change, P value) of the OAG group (Table 4) and the OHT group (Table 5). Following sustained IOP reduction in the OAG group, topographic parameters showing a statistically significant mean change were cup/disc ratio (-0.028, P=0.03), cup area (-0.061 mm^2, P=0.027), rim area (+0.060 mm^2, P=0.030), cup volume (-0.034 mm^3, P=0.030), mean cup depth (-0.021 mm, P=0.020), and maximum cup depth (-0.049 mm, P=0.039) and. In contrast, only one topographic parameter in the OHT group showed a statistically significant mean change. This was mean cup depth (-0.020 mm, P=0.022) [two-tailed paired t-test].

Table 8.6 compares the changes in topographic parameters between the OAG group and the OHT group following sustained IOP reduction using an analysis of variance (ANOVA). There was no statistically significant difference between the two groups in mean topographic change for all parameters ($P \geq 0.416$) [one-way ANOVA].

Evaluation of the topographic differences in individual eyes of each of the two study groups revealed a similar response to therapeutic IOP reduction in all parameters. For example, for the parameter mean cup depth, OAG and OHT patients showed identical topographic changes post-IOP reduction. Thirteen patients (65%) showed anterior displacement of the lamina cribrosa, 2 patients (10%) showed further posterior displacement and 5 patients (25%) showed no change (<10 microns difference). For maximum cup depth, of the 20 OAG patients that underwent therapeutic IOP

reductions, 12 patients (60%) showed anterior displacement, 6 patients (30%) showed further posterior displacement and 2 patients (10%) showed no change. Of the 20 OHT patients, 13 patients (65%) showed anterior displacement of the lamina cribrosa, while 5 patients (25%) showed further posterior displacement and 2 patients (10%) showed no change. Finally, for the parameter cup volume, of the 20 OAG patients that underwent therapeutic IOP reduction, 12 patients (60%) showed reduction of cup volume, 3 patients (15%) showed an increase and 5 patients (25%) showed no change. Of the 20 OHT patients, 11 patients (55%) showed reduction of cup volume, 4 patients (20%) showed an increase and 5 patients (25%) showed no change.

There was a considerable variation in the values of the mean topographic change within each study group indicating that some patients showed major changes while others did not. For example, for the parameter mean cup depth, this variation ranged between -0.090 mm and +0.041 mm in the OAG group and between -0.085 mm to +0.068 mm in the OHT group. For maximum cup depth this variation ranged between -0.268 mm and +0.090 mm in the OAG group and between -0.275 mm and +0.186 mm in the OHT group, while for the parameter cup volume, the variation ranged between -0.206 mm and +0.068 mm in the OAG group and between -0.200 mm to +0.070 mm in the OHT group.

Table 8.1: Patient Characteristics of the OAG and OHT Groups (Mean ± SD, P value)

	OAG GROUP (N = 20)	OHT GROUP (N = 20)	Significance
AGE (Yrs)	66.7 ± 10.9	57.2 ± 11.6	0.011
SEX (M/F)	9/11	9/11	
IOP PRE	22.2 ± 4.2	28.7 ± 3.9	0.000
IOP MAX	28.8 ± 6.2	30.3 ± 3.3	0.376
C/D RATIO	0.75 ± 0.2	0.41 ± 0.2	0.000
M.D.	-9.94 ± 8.3	-0.38 ± 2.4	0.000
REFRACT.	-0.94 ± 2.7	+0.23 ± 2.8	0.186
OPP	43.2 ± 6.1	42.8 ± 10.6	0.894

OAG = open angle glaucoma; OHT = ocular hypertension; IOP Pre = intraocular pressure prior to reduction; IOP Max = maximum recorded intraocular pressure; C/D = cup/disc ratio, M.D. = mean defect of visual field; Refr. = error of refraction; Perf. Pr. = calculated ocular perfusion pressure.

Two-tailed Student's t-test

Table 8.2: Patient Characteristics of the OAG and OHT Groups (Mean ± SD, P value)

		OAG GROUP (N = 20)	OHT GROUP (N = 20)	Significance
METHOD OF IOP REDUCTION	MED	4	12	
	LAS	10	8	
	SUR	6	0	
IOP POST		14.2 ± 4.7	19.0 ± 2.5	0.000
% RED'N		36.9 ± 16.1	32.7 ± 11.5	0.349
DURATN		14.2 ± 6.1	14.6 ± 6.3	0.827

OAG = open angle glaucoma; OHT = ocular hypertension; Med. = medical therapy; Las. = argon laser trabeculoplasty; Sur. = surgery (trabeculectomy); IOP Post = intraocular pressure after reduction; % Red'n= Mean percentage reduction of IOP; Duration = Mean number of weeks between reduction of IOP and the second session of SLDF.

Two-tailed Student's t-test.

Table 8.3: Baseline optic disc topographic parameters in OAG patients and OHT patients (Mean ± SD)

HRT Parameter	OHT	OAG	% Difference	P Value
Cup Area (mm^2)	0.568 ± 0.321	1.111 ± 0.412	+95.6	0.000
Cup/Disc Ratio	0.304 ± 0.167	0.512 ± 0.151	+68.4	0.000
Rim Area (mm^2)	1.301 ± 0.362	1.024 ± 0.307	-21.3	0.014
Cup Volume (mm^3)	0.175 ± 0.169	0.274 ± 0.158	+56.6	0.067
Rim Volume (mm^3)	0.358 ± 0.210	0.204 ± 0.092	-43.0	0.006
Mn. Cup Depth	0.266 ± 0.129	0.283 ± 0.089	+6.39	0.617
Mx. Cup Depth	0.685 ± 0.292	0.642 ± 0.201	-6.28	0.594
Cup Shape	-0.170±0.071	-0.081±0.084	-52.4	0.001
Height in Contour	0.415 ± 0.121	0.365 ± 0.222	-12.0	0.387

Two Tailed Student's T-Test ($P< 0.05$)

Table 8.4: Changes in optic disc topographic parameters after sustained IOP reduction in OAG patients (Mean ± SD)

HRT Parameter	Before IOP Reduction	After IOP Reduction	Unit Change	% Change	P Value
Cup Area (mm^2)	1.111 ± 0.412	1.050 ± 0.431	-0.061	-5.5	0.03
Cup/Disc Ratio	0.512 ± 0.151	0.484 ± 0.161	-0.028	-5.5	0.03
Rim Area (mm^2)	1.024 ± 0.307	1.084 ± 0.342	+0.060	+5.9	0.03
Cup Volume	0.274 ± 0.158	0.240 ± 0.158	-0.034	-12.4	0.03
Rim Volume	0.204 ± 0.092	0.216 ± 0.083	+0.012	+5.9	0.40
Mn. Cup Depth	0.283 ± 0.089	0.262 ± 0.083	-0.021	-7.4	0.02
Mx. Cup Depth	0.642 ± 0.201	0.593 ± 0.175	-0.049	-7.6	0.04
Cup Shape	-0.081 ± 0.084	-0.087 ± 0.080	-0.006	-7.4	0.29
Height in Contour	0.365 ± 0.222	0.332 ± 0.089	-0.033	-9.0	0.49

Two Tailed Paired T-Test ($P< 0.05$)

Table 8.5: Changes in optic disc topographic parameters after sustained IOP reduction in OHT patients (Mean ± SD)

HRT Parameter	Before IOP Reduction	After IOP Reduction	Unit Change	% Change	P Value
Cup Area (mm^2)	0.568 ± 0.321	0.517 ± 0.310	-0.051	-9.0	0.18
Cup/Disc Ratio	0.304 ± 0.167	0.271 ± 0.138	-0.033	-10.8	0.21
Rim Area (mm^2)	1.301 ± 0.362	1.352 ± 0.302	+0.051	+3.9	0.18
Cup Volume	0.175 ± 0.169	0.155 ± 0.153	-0.020	-11.4	0.09
Rim Volume	0.358 ± 0.210	0.370 ± 0.197	+0.012	+3.4	0.51
Mn. Cup Depth	0.266 ± 0.129	0.246 ± 0.120	-0.020	-7.5	0.02
Mx. Cup Depth	0.685 ± 0.292	0.656 ± 0.284	-0.029	-4.2	0.21
Cup Shape	-0.170 ± 0.071	-0.179 ± 0.075	-0.009	-5.3	0.18
Height in Contour	0.415 ± 0.121	0.414 ± 0.114	-0.001	-0.2	0.95

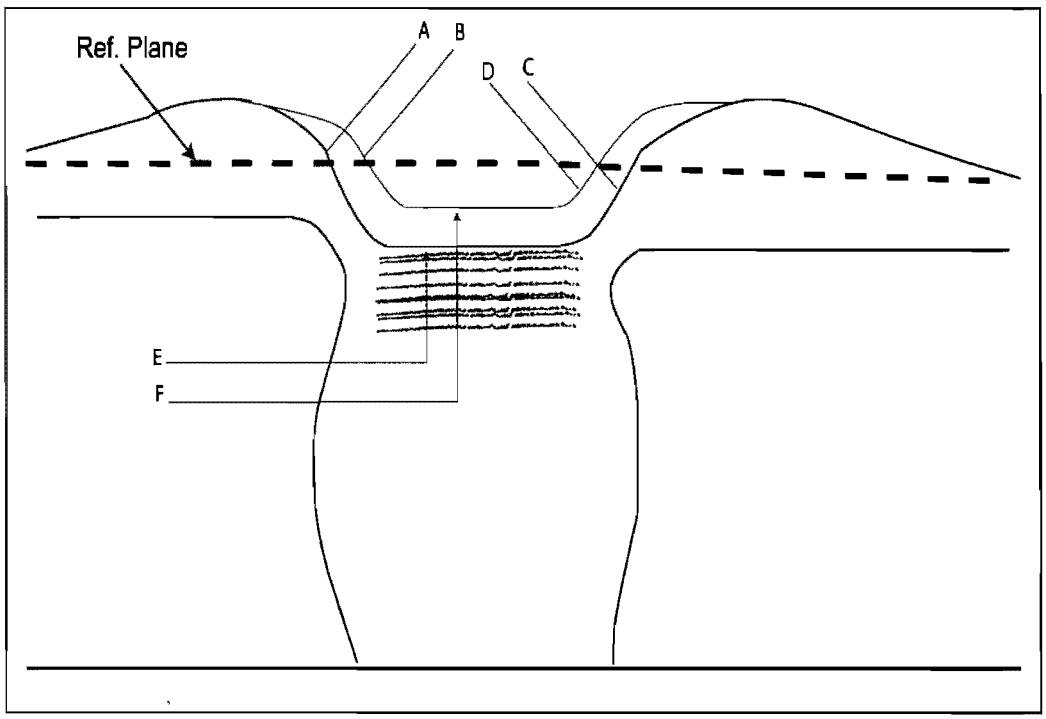
Two Tailed Paired T-Test ($P< 0.05$)

Table 8.6: Changes of topographic parameters in OAG patients versus OHT patients following sustained IOP reduction (Mean ± SD)

HRT Parameter	OHT	OAG	P Value
Cup Area (mm^2)	-0.055 ± 0.160	-0.058 ± 0.108	0.843
Cup/Disc Ratio	-0.036 ± 0.113	-0.027 ± 0.050	0.817
Rim Area (mm^2)	0.055 ± 0.160	0.057 ± 0.109	0.861
Cup Volume ($mm^{3)}$	-0.024 ± 0.048	-0.031 ± 0.060	0.439
Rim Volume (mm^3)	0.015 ± 0.074	0.010 ± 0.060	0.968
Mn Cup Depth (mm)	-0.021 ± 0.034	-0.021 ± 0.036	0.729
Mx Cup Depth (mm)	-0.039 ± 0.097	-0.050 ± 0.093	0.546
Cup Shape	-0.008 ± 0.029	-0.008 ± 0.022	0.887
Height in Contour (mm)	0.001 ± 0.065	-0.037 ± 0.194	0.476

ANOVA, ($P < 0.05$)

Figure 8.1:



Model of Topographical ONH changes with IOP reduction.

Ref. Plane: Reference Plane

A and B: Position of the neuroretinal rim surface before (A) and after (B) IOP reduction.

C and D: Position of outer limit of the cup (inner limit of the neuroretinal tissue below the reference plane) before (C) and after (D) IOP reduction.

E and F: Position of the lamina cribrosa surface before (E) and after (F) IOP reduction.

DISCUSSION

As glaucoma progresses, the lamina cribrosa becomes more exposed at the base of the cup of the optic disc. It also bows backwards, enlarges the size of some of its pores, and eventually becomes less compliant (more mechanically rigid) (41). As it permits the exit of one million axons and allows the entry of countless elements of microvasculature while simultaneously acting as a pressure barrier between intraocular and extraocular tissues, the lamina cribrosa has often been viewed as an anatomically vulnerable site in the pathophysiology of glaucoma. In experimental glaucoma, axoplasmic flow is interrupted at the level of the lamina cribrosa (42). The fact that the pathognomonic finding of glaucoma i.e. cupping, occurs in the optic nerve head reinforces the notion that the lamina cribrosa is a central actor in the disease process.

Altered lamina cribrosa compliance has long been postulated to play a role in the development of OAG. Studies suggest that there may be an initial hypercompliance in early experimental primate glaucoma (27,31) followed by a reduced compliance (i.e. increased rigidity) later in the course of the disease (21-28,30,31,43-44). Most investigators have hypothesized that reduced compliance was contributing to the development of glaucoma (although there was no direct evidence for this).

Studies have also suggested that lamina cribrosa compliance can be readily estimated using CSLO, by examining the position of the base of the cup relative to the retinal surface following IOP changes (3-5,30). The key parameter, which is closely linked to

lamina cribrosa position in almost all glaucoma patients is the mean cup depth as measured by CSLO (3-5,45). Several reports document that the position of the base of the cup or the lamina cribrosa moves anteriorly to a variable degree when IOP is reduced in glaucoma patients (3-5). However, the present report is the first that compares such changes in OAG and OHT patients.

The present study reports a significant difference in most baseline topographic parameters between the OAG and the OHT groups except for mean cup depth (P=0.617), maximum cup depth (P=0.594) and height in contour (P=0.387). A marginally significant difference was demonstrated in cup volume (P=0.067) [two-tailed Student's t-test]. Similar findings were reported by Zangwill et al (46) and Iester et al (17) who demonstrated that glaucomatous discs were different from ocular hypertension discs analyzed using a confocal scanning laser ophthalmoscope even when controlled for age (46) or disc size (17).

Following a sustained IOP reduction of 37%, the study reports a significant mean change in most topographic parameters in the OAG group. In the OHT group, and following an IOP reduction of 33%, only mean cup depth showed a significant mean change (P=0.02, two-tailed paired t-test). However, post-reduction unit change and % change in most topographic parameters were comparable between both OAG and OHT groups. None of the topographic differences between the two study groups showed a statistically significant difference ($P \geq 0.439$, ANOVA). These findings demonstrate that

the structural compliance of the ONH and lamina cribrosa in both OAG and OHT was similar even though only the OAG group shows the established disease process. There are two possible interpretations for this finding: either that compliance does not change in moderate OAG, or that those changes occur early and were already present in our population of OHT patients. We believe that at the stage of OAG patients enrolled in this study (C/D ratio 0.75 and MD -9.9), it is most likely that the compliance has already been altered. Therefore, our data suggests that compliance changes in the ONH and lamina cribrosa occur very early in the course of the disease. Moreover, the OHT patients selected for this study all had clinically-indicated IOP reduction. In other words, they had sustained ocular hypertension, sometimes accompanied by suspect (though obviously not glaucomatous) optic discs, suggesting that many of them had the earliest stage of the disease.

This supports previous reports from Bellezza et al (37) and Burgoyne et al (38) who attempted to characterize the mechanical properties of the ONH by studying the behavior of the connective tissue of the lamina cribrosa, scleral canal wall and peripapillary sclera in response to the mechanical stress generated by varying levels of IOP. The authors reported deformation of the lamina cribrosa in young monkeys with early experimental glaucoma and attributed their findings to damage to the ONH load-bearing connective tissue occurring early in the disease process. These findings were confirmed using both serial histological sections and high-resolution digital 3-D reconstruction of the connective tissues of the monkey's ONH.

There was a significant difference between our two study groups in terms of disc size (2.135 mm for the OAG group versus 1.869 mm for the OHT group, P=0.018 (two-tailed Student's t-test). To examine whether this disparity influenced the results we matched fourteen pairs of subjects from the two study groups for disc size (mean: 1.980 ± 30 mm). The OAG subgroup had a mean IOP reduction of 39% while OHT subgroup had a mean IOP reduction of 33%. None of the topographic differences between the two groups showed a statistically significant difference ($P \geq 0.093$, ANOVA). Topographic difference (mean ± SD) in mean cup depth was -0.025±0.036 mm in the OAG group compared to -0.010±0.031 mm in the OHT subgroup (P=0.235), while maximum cup depth showed a topographic difference of -0.056±0.095 mm in the OAG group compared to -0.006±0.093 mm in the OHT subgroup (P=0.173)[one-way ANOVA].

There was also a significant difference between our two study groups in terms of age, clinical C/D ratio as well as post-reduction IOP ($P \leq 0.011$) (Tables 1 and 2). These factors could have influenced the IOP dependant ONH topographic changes. By matching the two study groups for each of these parameters, none of the topographic differences were found to demonstrate a statistically significant difference ($P \geq 0.241$ for age), ($P \geq 0.314$ for clinical C/D ratio) and ($P \geq 0.243$ for post-reduction IOP) [one-way ANOVA].

We also examined the two study groups for the influence of percentage IOP reduction on topographic differences. A comparison between OAG and OHT patients with higher

percentage IOP reduction (>35%) was performed. None of the topographic differences between the two study groups showed a statistically significant difference (P≥0.362). Similarly, a comparison between OAG and OHT patients with lower % IOP reduction (<35%) showed no statistically significant difference (P≥0.206). Comparing the parameter mean cup depth in both OAG and OHT patients with higher percentage IOP reduction (>35%, N=10 for each subgroup) showed a topographic difference of -0.023 mm in the OAG and -0.025 mm in the OHT (P=0.914). A similar comparison between OAG and OHT patients with lower percentage IOP reduction (<35%, N=10 for each subgroup) showed a topographic difference of -0.023 mm in the OAG and -0.013 mm in the OHT (P=0.516) [one-way ANOVA].

Simple modeling of IOP-dependant volumetric changes in the base of the ONH:-

An unresolved issue is to what degree reversal of cupping represents forward displacement of the underlying lamina cribrosa as opposed to true increased volume of neuroretinal rim tissue. To approach this question, we used the data in Table 8.6 to model volumetric changes in the ONH. As seen in Figure 8.1, increased volumes to the neuroretinal rim that occur above the HRT reference plane are designated by the HRT software as increased rim volumes. Increased volumes to the neuroretinal rim below the HRT reference plane are designated as decreased cup volumes. Decreased cup volumes also include changes due to shallowing of the cup, caused primarily by anterior displacement of the lamina cribrosa.

In the case of the OHT group, the mean total of increased rim volume and decreased cup volume is $0.015mm^3 + 0.024mm^3 = 0.039mm^3 = 39.0 \times 10^6 \mu m^3$. The total lamina cribrosa volumetric displacement can be estimated by multiplying the mean change in cup depth by the mean disc area. This assumes that laminar displacement is uniform across its breadth, or at least that the mean displacement in the peripheral lamina (hidden behind neuroretinal rim) is the same as the mean of that measured centrally by the HRT. If peripheral lamina displaces less than central lamina, total lamina displacement will be overestimated. In the OHT group, the mean change in cup depth was 0.021 mm =21 μm. The mean disc area is the cup area plus the rim area, i.e. 0.568 +1.301 = 1.869 mm^2 = 1.869 x 10^6 μm^2. Estimated total laminar displacement is therefore 21 μm x 1.869 x 10^6 μm^2 = 39.2 x 10^6 μm^3, = 0.039 mm^3, identical to the measured volume for neuroretinal rim displacement.

Using the corresponding values in the OAG group, the mean total of increased rim volume and decreased cup volume is 0.041 mm^3 while the estimated mean laminar displacement is 0.045 mm^3. From these calculations we conclude that most if not all of the observed increases in neuroretinal rim volume can be attributed to the underlying displacement of the lamina cribrosa, rather than to increased volume of neuroretinal rim tissue.

The pressure-dependant ONH topographical changes observed in this study suggest that changes to laminar compliance occur early in the course of the disease. More detailed studies are required to confirm this, looking at cohorts of both early and moderate

glaucoma patients, as well as ocular hypertensives and normal subjects. To the best of our knowledge there exists no *in vivo* studies on changes in lamina cribrosa compliance with the development of glaucoma in humans and no studies examining the significance of such changes on the maintenance of visual field, the ultimate measure of function.

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Chapter 9

Relationship between Glaucomatous Visual Field Progression, Vasospasticity, and Changes in Optic Nerve Head Topography and Blood Flow at the time of Initial IOP Reduction: A Prospective Pilot Study

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ABSTRACT

Introduction: Recent published data from our laboratory suggests that, compared to those with thicker corneas, open angle glaucoma (OAG) and ocular hypertension (OHT) patients with thinner corneas had larger reductions in optic nerve head (ONH) cup depth and smaller improvements in neuroretinal rim blood flow when undergoing a sustained intraocular pressure (IOP) reduction. We investigated whether such ONH changes correlated with long term visual field progression.

Methods: Ten OAG, and 16 OHT (1 pre-perimetric glaucoma) patients had Heidelberg retina tomography (HRT) and scanning laser Doppler flowmetry (SLDF) of the ONH before and 2-6 months following therapeutically-indicated sustained IOP reduction. Peripheral vasospasticity was measured using finger Doppler flowmetry during cold water immersion. Ultrasonic pachymetry was performed. Visual field stability was then monitored over the following 3.5 ± 1.0 years using modified Hodapp-Anderson-Parrish criteria.

Results: Eight patients progressed (3 OHT and 5 OAG), 16 were stable (12 OHT and 4 OAG), 2 were indeterminate and excluded from further analyses (1 OHT and 1 OAG). At initial IOP reduction, patients that eventually progressed had 0.089±0.144 mm mean shallowing of maximum cup depth vs. 0.001±0.07 mm in the stable group. This did not reach statistical significance in the univariate analysis (P=0.137), but was significant in the multivariate analysis (P=0.005). Progressing patients were also significantly more

vasospastic (minimum finger blood flow 3.8±2.3 ml/mm^3 vs. 11.1±8.4 ml/mm^3, P=0.006). Progressing patients had non-significantly thinner corneas (546±50µm vs. 568±55µm, P=0.39) and non-significantly smaller initial increases in neuroretinal rim blood flow than non-progressing patients (32±97 au vs. 87±132 au, P=0.31).

Both groups had similar percentage IOP reduction (P=0.5), age (P=0.2), refractive error (P=0.2), ocular perfusion pressure (P=0.2) and mean follow-up period in months (P=0.9). The progressing group had lower IOPs both pre (P=0.012) and post (P=0.006) therapy, and worse visual field mean defect (P=0.047), smaller rim area before IOP reduction (P=0.03) and smaller mean retinal nerve fiber layer thickness and area both at baseline (P=0.01) and post IOP reduction (P<0.03) compared to the stable group.

Conclusion: In this pilot series, vasospasticity appears to be linked to an increased risk of glaucomatous visual field progression. Greater movement of the base of the cup, possibly interpreted as a sign of a more compliant lamina cribrosa, may also be linked to an increased risk of progression in glaucoma.

Key Words:

Visual Field change - OAG - OHT - ONH blood flow - ONH topography - Finger blood flow.

INTRODUCTION

In recent years the pathogenesis of glaucoma has been the focus of systematic ophthalmologic research. This has been precipitated by changes in the definition of glaucoma and the current belief that it has a multifactorial etiology. Elevated intraocular pressure (IOP) remains its most important risk factor (1), although mechanical factors of the optic nerve head (ONH) and vascular factors (2,3) are also gaining significance.

Topographic changes of the ONH are important clinical signs for diagnosis of glaucomatous optic neuropathy as well as progression monitoring. Studies demonstrated that the ONH may be deformed and is topographically susceptible to IOP changes, both *ex vivo* (4-11) and *in vivo* (12-17). Bellezza et al. (18) and Burgoyne et al. (19), among others, showed that deformations of the lamina cribrosa and the anterior scleral canal wall are the main cause of the observed ONH topographic changes. Such deformations were attributed to damage to the connective tissue of the ONH (20).

Quantitative assessment of pressure-dependant positional changes of the cup base relative to the retinal surface allows an estimation of lamina cribrosa compliance and can be measured using confocal scanning laser ophthalmoscopy (CSLO) (5,12,16). Based on these studies and others, the biomechanical compliance of optic nerve head (ONH) tissues has thus been hypothesized as a possible contributing factor to the susceptibility of some eyes to damage by glaucomatous optic neuropathy (4-9,11,21-24).

Vascular factors, including reduced ocular blood flow (25-28), systemic blood pressure alterations (29-31) and vasospasm (32-38), have also been reported to contribute to the pathogenesis of glaucoma. Recent technical advances have enabled research into the impact of such factors on ONH blood flow and autoregulation.

Autoregulation helps to maintain constant blood flow in the ONH, regardless of changes in ocular perfusion pressure (OPP) (OPP is defined as the mean arterial pressure of the ocular blood vessels minus the IOP). It has been suggested that defective autoregulation of the ONH may contribute to glaucomatous optic neuropathy (39,40), a hypothesis that was supported by a study from our laboratory (41) suggesting that autoregulation of ONH blood flow was abnormal in glaucoma patients. Such results have been also confirmed by others (42). Furthermore, peripheral vasospasm was reported to underlie such a defect in autoregulation (43,44). A high prevalence of peripheral vasospasticity has been reported in glaucoma patients. Phelps and Corbet (37) found a strong association between NTG and migrainous headaches while Drance et al (38) reported that 65% of NTG patients show a positive vasospastic response. These findings were later supported by results from the Collaborative Normal Tension Glaucoma Study (45) that demonstrated a 2.58-fold increased risk of progression in glaucoma patients suffering from migraines.

The Ocular Hypertension Treatment Study reported that central corneal thickness (CCT) is the strongest predictive factor for progression of OHT patients to glaucoma (46). Medeiros et al reported significantly thinner CCT in OHT patients with visual field loss, compared to OHT patients without visual field deficits (47,48) and suggested that CCT is a risk factor for visual field loss in pre-perimetric glaucoma (49). Herndon et al (50) showed that CCT is also correlated to the glaucoma severity at initial examination of glaucomatous patients and although the Early Manifest Glaucoma Trial (EMGT)(51) did not find a correlation between CCT and progressive visual field loss progression, Kim et al (52) found that thinner corneas were associated with greater visual field deterioration. Recently we studied the relationship between CCT and pressure sensitive changes of optic nerve topography and blood flow and demonstrated that OAG and OHT patients with thinner corneas had greater changes in the position of the base of the cup and lesser increases in neuroretinal rim blood flow when the IOP was reduced, suggesting a link between the mechanical and vascular aspects of the optic nerve head (53).

In this pilot study we explore the relationship between glaucomatous visual field progression and such factors as vasospasticity and changes in ONH topography and blood flow at the time of initial IOP reduction in a subgroup of patients that were previously studied (41). We also look at the possible correlation with CCT measurements.

PATIENTS AND METHODS

The study was approved by the research committee of Maisonneuve-Rosemont Hospital, University of Montreal. Informed consent was obtained from each patient before enrollment.

Twenty six patients, 16 OHT and 10 OAG, were recruited from the glaucoma clinics of the hospital into this study. OAG patients had gonioscopically open angles and fulfilled at least two of the following three criteria: a history of IOP greater than 21 mmHg, characteristic nerve fiber bundle visual field defects and glaucomatous optic neuropathy. OHT patients had a history of IOP greater than 24 mmHg on at least two occasions, normal visual fields and normal optic nerve head appearance based on slit-lamp biomicroscopy. Only one OHT subject had pre-perimetric OAG, with no visual field loss on standard automated perimetry and a suspicious optic nerve head with a cup-to-disc ratio of 0.7.

In all subjects, the eye with the higher baseline IOP was chosen for the study, or the right eye in case of equal IOP in both eyes. Subjects were excluded if any abnormal ocular findings were present other than pseudophakia, if significant media opacities precluded imaging, or if they were unable to cooperate.

Medical and ocular history, were recorded and IOP, best corrected visual acuity, and refractive errors were measured prior to the baseline study visit. A basic ophthalmologic

examination, including biomicroscopy, ophthalmoscopy and goniosocopy was performed and the visual field (VF) was assessed using automated perimetry (Humphrey Field Analyzer, Program 24-2, Humphrey Instruments, San Leandro, California).

Central corneal thickness (CCT) was measured, using an ultrasound pachymeter (DGH model 500). Five measurements were made perpendicular to the central cornea and the mean of the three closest numbers was used as the CCT. Systemic blood pressure and heart rate were recorded and ocular perfusion pressure was calculated as follows: {Ocular Perfusion Pressure = 2/3 [diastolic blood pressure + 1/3 (systolic blood pressure – diastolic blood pressure)] – IOP} (54). After the initial evaluation patients underwent baseline ONH blood flow and topography measurements.

The Scanning Laser Doppler Flowmeter (SLDF) used in this study (Heidelberg Retina Flowmeter - HRF, Heidelberg Engineering, GmbH, Heidelberg, Germany) is a non-invasive instrument combining laser Doppler flowmeter and scanning laser technology. It uses an infrared diode laser (780 nm wavelength) to measure backscattered light at different locations in the tissue of interest. A two-dimensional map of microvascular perfusion of the studied area (2.7mm x 0.7mm) is thus generated. Technical details related to the instrument are discussed elsewhere (55). Automatic full-field perfusion image analysis (AFFPIA) software developed by Michelson and associates (56) was used for analysis of the perfusion images. A recent study from our laboratory describes

our method of SLDF full-field perfusion analysis and demonstrates its highly reproducible inter- and intra-session measurements (57). All images were reviewed by the same observer prior to data analysis and patients whose images were considered to be of poor quality or unsuitable for analysis were excluded from the study. AFFPIA was performed on each of the five images with measurements of Flow given in arbitrary units (au) for the neuroretinal rim area, temporal and nasal peripapillary retinal areas. Data presented for each anatomical area represents the mean value from 5 perfusion images.

Topography of the ONH was assessed using the Confocal Scanning Laser Ophthalmoscope (Heidelberg Retina Tomograph (HRT), Heidelberg Engineering, GmbH, Heidelberg, Germany) (58,59). The HRT employs a scanning ophthalmoscope with an infrared diode laser of wavelength 670 nm and confocal optics for image acquisition. The depth of each topographic image series ranges from 0.5mm to 4.0mm depending on ONH morphology. Using a 10-degree field and the appropriate focus and depth settings, a total of 5 images were acquired sequentially. All images were reviewed by the same observer and prior to data analysis the best three images in terms of focusing, centration and brightness were chosen. A mean topography image was then created from these three images. The optic disc margin contour line was drawn on the Pre-IOP reduction mean topography image while viewing stereo photographs of the ONH.

The HRT parameters investigated in this study were cup area, cup/disc area ratio, rim area, mean cup depth, maximum cup depth, cup volume, rim volume, cup shape measure (CSM), height variation contour (HVC), retinal nerve fiber layer thickness (RNFLT) and cross-section area (RNFLA). These parameters were measured relative to a standard reference plane, defined as 50 microns below the average height of the contour line in an inferior temporal segment between 350 degrees and 356 degrees.

Following baseline measurements all patients underwent IOP reduction by medical, laser, or surgical intervention. All patients included in the study had sustained IOP reduction of 20% or more, as well as a minimum of four weeks follow-up between the intervention and the second imaging session. Patients who underwent filtration surgery had at least 12 weeks follow-up following the intervention to avoid changes related to hypotony, edema or inflammation. None of the study patients had hypotony or hypotony-related ONH or macular changes.

Patients then underwent a second session of HRT and HRF imaging, using the same settings as previously used for image acquisition and for automatic full-field perfusion image analysis. The best three images for HRT and the best five images for SLDF were similarly chosen and a mean topography and mean flow readings were obtained respectively for both baseline and follow-up sessions. The optic disc margin contour line was then exported from the Pre-IOP reduction mean topography image to the Post-IOP reduction mean topography image using the capabilities of the HRT.

Finger blood flow measurements were obtained using the Transonic laser Doppler Flowmeter (Transonic Systems Inc., Ithaca, NY). Technical details related to the instrument and technique are discussed elsewhere (44). Following the protocol presented by Drance et al (38), baseline flow was measured on the underside of the end of the middle finger of a randomly selected hand. After a stable flow reading was obtained the hand was immersed in warm water (40°C) for 2 minutes (Fmax). The hand was then immersed in ice-cold water (4°C) for 10 seconds (Fmin) and then finally placed at room temperature for 10 minutes (recovery). Finger flow measurements were made continuously by the laser Doppler flowmeter and transmitted in real-time to a computer via an interface. Blood pressure measurements were taken while recording flow at baseline, exposure to warm and recovery. A vasospastic response was taken as present when the vasospasticity index (ratio of maximum flow to minimum flow) exceeded 7, i.e. (Fmax/Fmin > 7). The absolute level of Fmin was also indicative of vasospasticity when it is depressed (38).

Visual Field (VF) stability was monitored over a period of 3.5 ± 1.0 years, by retrospectively reviewing available Humphrey 24-2 Visual Field Analyzer tests of the study group. This was performed by specially trained research members and confirmed by glaucoma-trained specialists, using specific pre-determined progression criteria for VF loss.

Since some fields were 24-2 full threshold and others were 24-2 SITA standard, and since glaucoma change probability analysis was not available for most patients, we

adopted a more general approach to assessment of visual field progression. Thus, VF progression was assessed using modified Hodapp-Anderson-Parrish VF Progression criteria (60). Suspected progression was defined as either:

1- Cluster of three or more non-edge points that decreased by 5 dB or more compared to the previous measurement, or

2- Single non-edge point that decreased by 10 dB or more compared to the previous measurement.

An exception to the non-edge rule were nasal edge points, immediately adjacent to horizontal midline, since glaucomatous VF loss has been particularly linked to such points (61). If the aforementioned decreases were reproduced in two out of three consecutive VF tests, then the patient was characterized as having Confirmed Progression and not Suspected Progression.

Topographic and ONH blood flow parameters before and after IOP reduction were then compared in the progressed and non-progressed groups in a univariate fashion (Student's t-test). A multivariate model, taking into account changes from pre to post IOP reduction in five hypothesized risk factors for glaucomatous VF progression, i.e. CCT, ONH blood flow, mean cup depth, maximum cup depth and Flow min, was analyzed. The latter data was also analyzed in a univariate fashion based on two–tailed Student's t-tests. Statistical significance was set at $P<0.05$.

RESULTS

Twenty six patients (16 OHT and 10 OAG) were monitored for VF progression over a period of 3.5±1.0 years (range: 0.7-4.8 years). Pre and post-IOP reduction ONH topography and flowmetry were performed and good quality images were obtained for all patients. Eight patients progressed (31%), 3 OHT (12%) and 5 OAG (19%). Sixteen patients were stable (61%), 12 OHT (46%) and 4 OAG (15%). Two patients (8%) were indeterminate (having suspected progression on their last visual field but no confirmatory field available) and were excluded from the analysis (1 OHT and 1 OAG). Data is thus presented for 24 patients (15 OHT and 9 OAG).

Nine patients had a history of hypertension (2 progressed), five patients had thyroid problems (4 progressed), six patients had a history of heart disease (3 progressed), and 4 patients had diabetes mellitus (1 progressed). Some patients had a combination of the above pathologies.

In this study, the measured reference plane height for the HRT images did not change significantly between pre and post-IOP reduction images. Average reference plane height was $0.332 \pm 0.127 mm^2$ and $0.324 \pm 0.117 mm^2$ respectively (P=0.83, two-tailed paired t-test).

Table 9.1 summarizes the characteristics of the progressed and non-progressed groups and their statistical significance, including age, gender, OHT to OAG ratio, IOP

measurements, as well as VF mean defect. There was no statistically significant difference between the two groups in the means of age, refractive error, maximum-recorded IOP and calculated ocular perfusion pressure ($P \geq 0.1$). Progressing patients also had insignificantly thinner corneas (546 ± 50 μm versus 568 ± 55 μm, P=0.39). Both groups had similar percentage IOP reduction (35 ± 12% in the progressed group and 31 ± 12% in the nonprogressed group, P=0.51) and similar duration between the IOP reduction and the 2nd imaging session (17 weeks versus 19 weeks, P=0.65). The progressed group demonstrated lower IOP both at baseline (P=0.012) and post-therapy (P=0.006) and a higher mean defect of visual field (P=0.047). There was no significant difference between the two study groups in the duration of monitoring for visual field stability (43 ± 12 months in the progressed versus 42 ± 13 months in the nonprogressed group, P=0.87).

Tables 9.2 and 9.3 compare baseline and post-IOP reduction HRT topographic parameters for the progressed and nonprogressed groups whereas Table 9.4 compares the unit change of such parameters and their statistical significance. A statistically significant difference was shown between the progressed and non progressed groups at baseline rim area (P=0.032) and at both baseline and post IOP reduction mean RNFL thickness and cross-sectional area ($P \leq 0.029$).

At initial therapeutic IOP reduction, patients that eventually progressed had 0.089 ± 0.144 mm shallowing of maximum cup depth versus 0.001 ± 0.05 mm in the non progressed group. This difference in maximum cup depth change from pre to post

therapy was non-significant in the univariate analysis (P=0.137). Mean cup depth showed a similar non-significant trend, with a shallowing of 0.024 ± 0.45 mm in the progressed group versus 0.011 ± 0.30 mm in the non progressed group (P=0.417).

Table 9.5 presents SLDF perfusion values in the two study groups. Mean neuroretinal rim blood flow at baseline and post-IOP reduction as well as rim flow change in arbitrary units (AU) and as % flow change are reported in both groups. Progressing patients had non-significantly smaller initial increases in neuroretinal rim blood flow than stable patients (32.3 ± 97.1 au versus 87.1 ± 132 au, P=0.31).

Table 9.6 contains information regarding the finger blood flow of the progressed and non progressed groups as measured by the Transonic laser Doppler Flowmeter. In univariate analysis, the mean minimal finger blood flow of the progressed group was found to be significantly smaller than in the stable group (3.8 ± 2.3 ml/mm^3 versus 11.1 ± 8.4 ml/mm^3, P=0.006). There was no significant difference between the two groups in the vasospasticity index (P=0.30).

All univariate analyses were "intent-to-treat" including all study patients in all analyses (N=24) and excluding data only from the specific tests for which they did not perform. A multivariate ANOVA model was thus established (including five hypothesized risk factors for glaucomatous VF progression (CCT, ONH rim blood flow, mean cup depth, maximum cup depth and minimum finger blood flow). It should be noted that there

were three patients excluded from the multivariate model, due to missing data for at least one of the parameters included in the model. These patients were not excluded from the univariate intent-to-treat analysis.

The ANOVA model thus included 21 patients (6 progressed and 15 non-progressed) and demonstrated no significant differences in CCT (P=0.81), mean cup depth change (P=0.22) and ONH rim blood flow change (P=0.46) between the progressed and non progressed groups.

Maximum cup depth showed a significant difference between the two groups with larger shallowing in the progressed group (-0.139 ± 0.126 mm versus -0.002 ± 0.073 mm; P=0.005). Minimum finger blood flow, the fifth risk factor included in the multivariate ANOVA, showed a strong trend for lesser values in the progressed group, with a marginally significant P value (4.05 ± 2.3 ml/mm^3 versus 11.1 ± 8.4 ml/mm^3, P=0.061).

A per-protocol univariate analysis was also performed including only the 21 patients that had complete data. In this analysis the significant difference in mean minimum finger blood flow value between the groups was reproduced (P=0.008). Maximum cup depth change from pre to post IOP reduction remained significantly greater in the progressed group compared to the stable group (P=0.005) whereas mean cup depth change remained non-significant (P=0.215). Changes in other topographic parameters and in neuroretinal rim blood flow were not different from the intent-to-treat univariate

analysis. Rim area remained smaller in the progressed group both at baseline (P=0.015) and at follow-up (P=0.062). Baseline and post IOP reduction mean RNFL thickness and cross-sectional area were significantly different ($P \leq 0.029$) and maximum cup depth post therapy was larger in the non-progressed group (0.688 ± 0.269 mm versus 0.465 ± 0.163 mm; P=0.076).

Table 9.1: Patient Characteristics of the study sample when grouped as progressed and non-progressed.

Parameter	Progressed (N=8)	Non-progressed (N=16)	P-value
Diagnosis OHT/OAG	3/5	12/4	
Gender Female/Male	6/2	7/9	P=0.15*
Age ± SD (Years)	66.0 ± 13.5	59.3 ± 10.8	P=0.20
VF Follow-up ± SD (Months)	42.5 ± 12.3	41.5 ± 13.4	P=0.87
IOP Maximum (mmHg) ± SD	25.3 ± 7.9	31.6 ± 7.1	P=0.06
IOP Pre-Therapy (mmHg) ± SD	21.5 ± 5.9	27.1 ± 4.0	P=0.012
IOP Post-Therapy (mmHg) ± SD	14.1 ± 4.2	18.3 ± 2.5	P=0.006
IOP Mean % Reduction ± SD	34.8 ± 11.7	31.3 ± 12.2	P=0.51
IOP Reduction Method	2 S / 1 M / 5 L	10 M / 6 L	P=0.02*
Duration ± SD (Weeks)	17.4 ± 5.2	19.5 ± 16.3	P=0.65
Refraction Error ± SD Dioptres	-0.84 ± 4.16	1.01 ± 2.48	P=0.19
Mean Defect of Vis. Field (dB) ± SD	-8.91 ± 9.87	-0.47 ± 2.83	P=0.05
Ocular Perf. Pressure (mmHg) ± SD	46.83 ± 8.16	41.87 ± 8.62	P=0.19
Central Corneal Thickness (μm) ± SD	546 ± 50	568 ± 55	P=0.39

OHT: ocular hypertension; OAG: open angle glaucoma; IOP: intraocular pressure; Duration: Mean number of weeks between IOP reduction and 2nd SLDF exam; VF Follow-up: period of visual field monitoring; M: medications; S: surgery; L: laser trabeculoplasty. All P values calculated using Student's two-tailed t-test, except those marked with *, which were calculated using Pearsons's Chi-square. Statistical significance set at $P < 0.05$.

Table 9.2: Baseline topographic parameters of the study sample (progressed and non-progressed groups).

Baseline HRT Parameters (N=24)	**Progressed (Mean ± SD)**	**Non-Progressed (Mean ± SD)**	**P-Value**
Disc Area (mm^2)	1.894 ± 0.358	1.988 ± 0.367	P=0.559
Cup Area (mm^2)	0.886 ± 0.472	0.639 ± 0.355	P=0.164
Cup/Disc Area Ratio	0.467 ± 0.234	0.311 ± 0.141	P=0.053
Rim Area (mm^2)	1.008 ± 0.434	1.349 ± 0.292	P=0.032
Cup Volume ($mm^{3)}$	0.181 ± 0.118	0.199 ± 0.197	P=0.80
Rim Volume (mm^3)	0.234 ± 0.163	0.378 ± 0.211	P=0.11
Mean Cup Depth (mm)	0.227 ± 0.092	0.280 ± 0.134	P=0.33
Max Cup Depth (mm)	0.579 ± 0.230	0.679 ± 0.275	P=0.39
Cup Shape Measure	-0.123 ± 0.07	-0.143 ± 0.059	P=0.46
Height Variation Contour (mm)	0.464 ± 0.310	0.414 ± 0.118	P=0.57
MRNFL thickness (mm)	0.139 ± 0.113	0.244 ± 0.079	P=0.014
MRNFL area (mm^2)	0.692 ± 0.540	1.219 ± 0.413	P=0.014

MRNFL: Mean retinal nerve fiber layer; SD: Standard Deviation. All P values calculated using Student's two-tailed t-test, with statistical significance set at $P < 0.05$.

Table 9.3: Post-IOP reduction topographic parameters of the study sample (progressed and non-progressed groups).

Post-IOP reduction HRT Parameters (N=26)	**Progressed (Mean ± SD)**	**Non-Progressed (Mean ± SD)**	**P-Value**
Cup Area (mm^2)	0.755 ± 0.517	0.632 ± 0.356	P=0.500
Cup/Disc Area Ratio	0.385 ± 0.228	0.306 ± 0.144	P=0.308
Rim Area (mm^2)	1.137 ± 0.407	1.356 ± 0.282	P=0.137
Cup Volume ($mm^{3)}$	0.148 ± 0.116	0.191 ± 0.181	P= 0.54
Rim Volume (mm^3)	0.266 ± 0.207	0.369 ± 0.187	P=0.231
Mean Cup Depth (mm)	0.203 ± 0.083	0.269 ± 0.122	P=0.185
Max. Cup Depth (mm)	0.490 ± 0.198	0.678 ± 0.263	P=0.089
Cup Shape Measure	-0.114 ± 0.071	-0.155 ± 0.070	P=0.196
Height Variation Contour (mm)	0.373 ± 0.118	0.405 ± 0.100	P=0.497
MRNFL thickness (mm)	0.166 ± 0.060	0.242 ± 0.081	P=0.029
MRNFL area (mm^2)	0.803 ± 0.284	1.204 ± 0.420	P=0.024

MRNFL: mean retinal nerve fiber layer; SD: standard deviation. All P values calculated using Student's two-tailed t-test, with statistical significance set at $P < 0.05$.

Table 9.4: Change of topographic parameters (Post-IOP minus baseline) in the progressed and non-progressed groups.

HRT Parameters Change (Post-Pre) (N=24)	**Progressed (Mean ± SD)**	**Non-Progressed (Mean ± SD)**	**P-Value**
Cup Area (mm^2)	-0.131 ± 0.243	-0.007 ± 0.061	P=0.197
Cup/Disc Area Ratio	-0.081 ± 0.173	-0.005 ± 0.031	P=0.253
Rim Area (mm^2)	0.129 ± 0.245	0.007 ± 0.061	P=0.206
Cup Volume ($mm^{3)}$	-0.033 ± 0.077	-0.008 ± 0.027	P=0.394
Rim Volume (mm^3)	0.032 ± 0.126	-0.008 ± 0.039	P=0.407
Mean Cup Depth (mm)	-0.024 ± 0.045	-0.011 ± 0.030	P=0.417
Max. Cup Depth (mm)	-0.089 ± 0.144	-0.001 ± 0.071	P=0.137
Cup Shape Measure	-0.002 ± 0.031	-0.011 ± 0.027	P=0.430
Height Variation Contour (mm)	-0.090 ± 0.292	0.009 ± 0.053	P=0.461
MRNFL thickness (mm)	0.027 ± 0.085	-0.003 ± 0.032	P=0.373
MRNFL area (mm^2)	0.111 ± 0.396	-0.016 ± 0.168	P=0.413

MRNFL: Mean retinal nerve fiber layer; SD: Standard Deviation. All P values calculated using Student's two-tailed t-test, with statistical significance set at $P < 0.05$.

Table 9.5: Optic nerve head blood flow data in progressed and non-progressed groups at baseline, follow-up, unit change and % change.

ONH Blood Flow (N=24)	**Progressed (Mean ± SD)**	**Non-Progressed (Mean ± SD)**	**P-Value**
Mean Rim Flow Pre Therapy (AU) ± SD	240.50 ± 224.21	234.62 ± 105.44	P=0.93
Mean Rim Flow Post Therapy (AU) ± SD	272.79 ± 184.07	321.72 ± 123.9	P=0.45
Mean Rim Flow Change Units ± SD	32.29 ± 97.08	87.10 ± 132.48	P=0.31
Mean Rim Flow Change %	45.01 ± 81.66 %	57.32 ± 78.39 %	P=0.72

SD: Standard Deviation; AU: arbitrary units; all P values calculated using Student's two-tailed t-test, with statistical significance set at $P<0.05$.

Table 9.6: Finger blood Flow data for the progressed and non-progressed groups.

Finger Doppler Flowmetry	**Progressed (N=7)**	**Non-Progressed (N=15)**	**P-value**
Mean Base flow ± SD	40 ± 22.2	42.9 ± 19.2	P=0.771
Mean Max. Flow ± SD	47.2 ± 16.6	54.6 ± 18.6	P=0.368
Mean Min. Flow ± SD	3.8 ± 2.3	11.1 ± 8.4	P=0.006
Mean Max/Min Flow ± SD	16.3 ± 9.3	11.3 ± 12.2	P=0.306
Vasospast./Non-vasospast.	6/1	9/6	

SD: Standard deviation; Max: maximal; Min: minimal; Max/Min: maximal to minimal flow ratio; all P values calculated using Student's two-tailed t-test, with statistical significance set at $P<0.05$.

DISCUSSION

Recent data from our lab (53) suggests that OAG and OHT patients with thinner corneas had larger reductions in cup depth following sustained IOP reduction which could be interpreted as larger lamina cribrosa movement. These patients also had smaller improvements in neuroretinal rim blood flow with IOP reduction. It can therefore be hypothesized that a thin cornea may be associated with thin sclera and a thin lamina cribrosa, and that a thin lamina cribrosa shows greater compliance to IOP changes rendering the ONH more susceptible to damage. Whether this finding, in turn, translates into greater progression of the disease was unknown and was thus the focus of this pilot study.

The current pilot study suggests that upon sustained IOP reduction, greater movement of the base of the cup (89 versus 1 microns, P=0.005, one-way ANOVA), interpreted as a sign of a more compliant lamina cribrosa, appears to be linked to an increased risk of progression in glaucoma. Vasospasticity (manifested as lower minimum finger blood flow, P=0.006, Student's two-tailed t-test) also appears to be strongly linked to an increased risk of progression. There were tendencies towards a smaller initial increase in rim blood flow (32 au versus 87 au, P=0.31, Student's two-tailed t-test) upon IOP reduction and towards thinner corneas (546 microns versus 567 microns; P=0.39, Student's two-tailed t-test) in progressive patients, but this association did not reach statistical significance.

Studies have demonstrated that ONH morphology (cupping) improves in most glaucoma patients following sustained therapeutic IOP reduction (12-14). Similar degrees of reversal of cupping were shown to occur in parallel in both glaucoma patients and ocular hypertensives suggesting that changes to lamina cribrosa compliance could occur early in the natural history of the disease (62).

In the current study, the majority of the discs within the progressed group showed an anterior displacement of the base of the cup. However, a number remained stable and some even changed in the opposite direction. This reflected in the larger values for standard deviations implying a wide range of individual variations within the group.

Altered lamina cribrosa compliance has long been postulated to play a role in the pathogenesis of glaucoma. Studies suggest that there may be an initial hypercompliance in early experimental glaucoma (23,26) followed by reduced compliance (i.e. increased rigidity) later in the course of the disease (4-11,16,21,63-65). Most investigators hypothesized that such *decreased* compliance contributed to the progression of the disease. However recent studies suggest that it is *increased* compliance that might be what really contributes to glaucomatous damage (62,66). Increased mechanical movement of the lamina cribrosa and the ONH tissues may play a causal role in glaucomatous optic neuropathy and visual field loss in two distinct ways. Through direct and repetitive mechanical compression of the nerve axons traversing the ONH, or

through indirect ischemic damage of the neurons, subsequent to mechanical compression of the vasculature of the optic nerve passing through the laminar sheets.

Anterior displacement of the position of the base of the cup or the lamina cribrosa has been consistently reported in response to sustained reduction of IOP in glaucoma patients and was found to correlate well with the percentage of IOP reduction (12,14). Such movement can be readily estimated using CSLO, by examining the position of the base of the cup relative to the retinal surface following IOP changes (12-14,16). However, the present report is the first that correlates such changes to long-term visual field stability over a period of 3.5 years.

This data contradicts the histological findings of Zeimer and Ogura (7) who reported decreased ONH compliance with worsening of the visual field. However, their studies were conducted on postmortem human glaucomatous eyes as opposed to glaucoma patients and ocular hypertensives.

We also studied the relationship between glaucomatous visual field progression and CCT. Several studies evaluated the link between CCT and the severity of glaucomatous ONH damage at presentation as well as the rate of visual field progression during follow-ups. Chauhan et al (67) reported no relationship between CCT and visual field or optic disc progression in glaucoma patients whereas Jonas et al (68) reported that CCT correlated well with the amount of optic nerve damage but not with the risk of visual field progression. The current study similarly could not report any significant difference

in CCT between eyes with progression and eyes with stable visual fields though progressing eyes did have slightly thinner corneas (P=0.39, two-tailed Student's t-test).

We also found that patients with progressive visual field loss show tendencies towards smaller improvements in ONH blood flow (P=0.31, two-tailed Student's t-test) following sustained IOP reduction. Reduced neuroretinal rim blood flow could be linked to more compliant ONHs with greater anterior displacement of the lamina cribrosa following IOP reduction. This anterior displacement may damage the ONH microvasculature when, due to diurnal or post-therapy IOP fluctuations, it repeats on a daily basis. Larger anterior displacement upon IOP reduction could also compress the lamina cribrosa plates and the microvasculature therein, leading to smaller improvements in ONH blood flow.

Vasospasticity also appeared to be strongly linked to an increased risk of progression in this pilot series. Numerous studies demonstrated that glaucoma patients have significantly increased incidence of peripheral vasospasm (38,69,70). Schulzer et al (43) reported that glaucoma patients could be divided into two groups: patients that were predominantly vasospastic and patients that had predominantly small vessel disease. In the vasospastic group there was a high positive correlation between the amount of visual field damage and the highest IOP while in those with small vessel disease, no such correlation was found. Previously presented data by our lab (44) demonstrated that ONH blood flow was more sensitive to IOP changes in vasospastic glaucoma patients.

This finding suggests that vasospastic phenomenon underlies reduced blood flow at baseline in these subjects and that reduction of IOP might relieve ONH vasospasm when present, resulting in greater increases in blood flow. Moreover, the Collaborative Normal-Tension Glaucoma Study (CNTGS) (51) found that while women with migraines benefited from IOP lowering, patients with a history of cardiovascular disease or a family history of stroke did not, once again linking vasospasticity, IOP, and progressive visual field loss.

The progression of glaucoma and the monitoring of deterioration of function in OAG patients has been a priority of research for quite some time. Although quite a few advances have been achieved in recording structural progress of the disease through the use of various retinal imaging techniques, the monitoring of functional progress can be harder, more subjective and less clearly definable. However it is evident that, to the patient, the actual structural deterioration will have no effect if it is not accompanied by worsening of his vision and therefore the assessment of visual field is an invaluable tool in the management of glaucomatous patients. To the best of our knowledge there have been no *in vivo* studies examining the significance of changes in lamina cribrosa compliance, vasospasticity or ONH blood flow over time or linking the effect of such changes on the maintenance of visual field, the ultimate measure of function.

Limitations for this study include the relatively small sample size in relation to the variables studied and the lack of a standardized computation for visual field progression

assessment. Additional long-term prospective studies should be conducted using a larger sample size and standardized software that will use more complex regression analysis to assess visual field progression.

The results of this study represent a possible means for identification and assessment of patients with higher risk for glaucoma progression and visual field loss. Due to technological imaging innovations patients with greater lamina cribrosa compliance may be readily identified using a simple CSLO examination before and after therapeutic IOP changes. Since these patients are at high risk for visual field damage, appropriate personalized treatment and more aggressive therapy may then be introduced to insure decreased morbidity. This pilot data, while far from conclusive, suggests that there may be interplay between the factors studied and disease progression. The data also underscores the proposed links between vasospasm, optic nerve blood flow and optic nerve compliance on the one hand, and the pathophysiology of glaucoma on the other hand.

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Chapter 10

Conclusion and Future Prospects

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The present study is unique because it is the first quantitative measurements of perfusion obtained directly from the neuroretinal rim tissue of OAG and OHT patients before and after therapeutic IOP reduction. The study is the *first* to report improvement in ONH blood flow in OAG patients with sustained IOP reduction and the first that links such changes to peripheral vasospasm.

The present studies are also one of the first to investigate *in vivo* changes in lamina cribrosa compliance in glaucoma patients and to look at its importance as a risk factor for progression of glaucoma aiming to explore the relationship between laminar compliance and ONH blood flow.

The study confirms previous reports as regards decreased baseline ONH blood flow in OAG patients compared to OHT patients and normal subjects (chapter 4). Ocular hypertensives with larger cup/disc ratios were shown to have lower neuroretinal rim blood flow than those with smaller cups. It was not established whether these changes precede or result from the disc changes but the findings suggest that a vascular defect occurs in high-risk OHT early in the pathogenesis of the disease and before the manifestation of visual field defects as assessed by standard automated perimetry.

This study demonstrates that, following similar percentage of therapeutic IOP reduction, blood flow improves in the neuroretinal rim of the ONH in glaucoma patients, while remains stable in most ocular hypertensives (chapter 5). This finding is a direct

demonstration of the positive impact of IOP therapy on optic nerve blood flow. The results were recently replicated in a new cohort of patients in our laboratory.

The observed changes can be attributed to defective autoregulation of the ONH blood flow in glaucoma while such autoregulation remained intact for most ocular hypertensives. Peripapillary retinal blood flow changes were not significant in OHT patients or in OAG patients suggesting an intact retinal autoregulation in both groups.

Our findings were obtained using SLDF images and automatic full-field perfusion image analysis (AFFPIA). We have studied the application of this technique as well as its intrasession and intersession reproducibility in detail (chapter 3). AFFPIA was shown to permit non-invasive, high resolution mapping of perfused tissues and capillaries of the ONH and peripapillary retina. Compared to the original HRF analysis technique, it significantly reduced variables caused by the patient, operator or device and permited highly reproducible quantification and documentation of the entire perfusion map including the neuroretinal rim in glaucoma patients. Obtaining mean values from at least three images was shown to improve both the intrasession and intersession reproducibility of this technique.

We went on to show that vasospastic OAG and OHT patients have greater improvements in neuroretinal rim blood flow following sustained IOP reduction compared to non-vasospastic subjects (chapter 6). This finding suggests that a

vasospastic phenomenon underlies reduced ONH blood flow at baseline in these subjects.

Our studies of ONH morphology in OAG and OHT patients has shown a significant difference in most baseline topographic parameters between the two groups except for mean cup depth and maximum cup depth (chapter 7). Following sustained IOP reduction change, cupping improved in most OAG patients. However, none of the topographic changes showed statistically significant difference between the two groups, which suggests that the structural compliance of the lamina cribrosa in our population of OAG and OHT was similar even though only the OAG group shows the established changes. A possible explanation would be that changes to laminar compliance in ocular hypertensives occur very early in the course of the disease.

We also have shown that the position of the lamina cribrosa moves anteriorly to a variable degree when IOP is reduced in glaucoma patients. Modelling of CSLO data from our patients shows that virtually all ONH pressure-dependant morphological changes, including those of the neuroretinal rim, are due to anterior displacement of the underlying lamina cribrosa.

The relationship between laminar compliance and central corneal thickness was also examined (chapter 8). Our data suggests that following similar percentage of sustained IOP reduction both OAG patients and OHT patients with thinner corneas show greater forward displacement of the lamina cribrosa compared to those with thicker corneas. Upon IOP reduction, patients with thinner corneas had smaller improvements of ONH

blood flow. The results suggest a link between a thin cornea and a thin sclera, which in turn could be linked to a thin lamina cribrosa. A thin and more compliant lamina may compress the laminar plates and the microvasculature therein, leading to smaller improvements in ONH blood flow.

Finally, we examined visual field progression over a period of 3.5 years in a pilot cohort of our population of glaucoma patients and ocular hypertensives (chapter 9). Progressive patients were more vasospastic and had thinner corneas compared to stable patients. Following IOP reduction, progressive patients also had greater forward displacement of the lamina cribrosa interpreted as a sign of a more compliant lamina cribrosa as well as smaller improvements in neuroretinal rim blood flow.

The pilot data suggests a model which shows the contribution of various factors to progression. In this model a thin cornea may be associated to a thin sclera and a thin lamina cribrosa, and a thin lamina cribrosa may be more flexible and thus more susceptible to IOP fluctuations whether diurnal or long-term. Movement of the lamina cribrosa can damage adjacent axons or ONH vasculature or render them more susceptible to damage. Laminar movement can, in addition, trigger vasospastic episodes (the way movements of the meninges can trigger migraines) which in turn can disrupt normal autoregulatory processes.

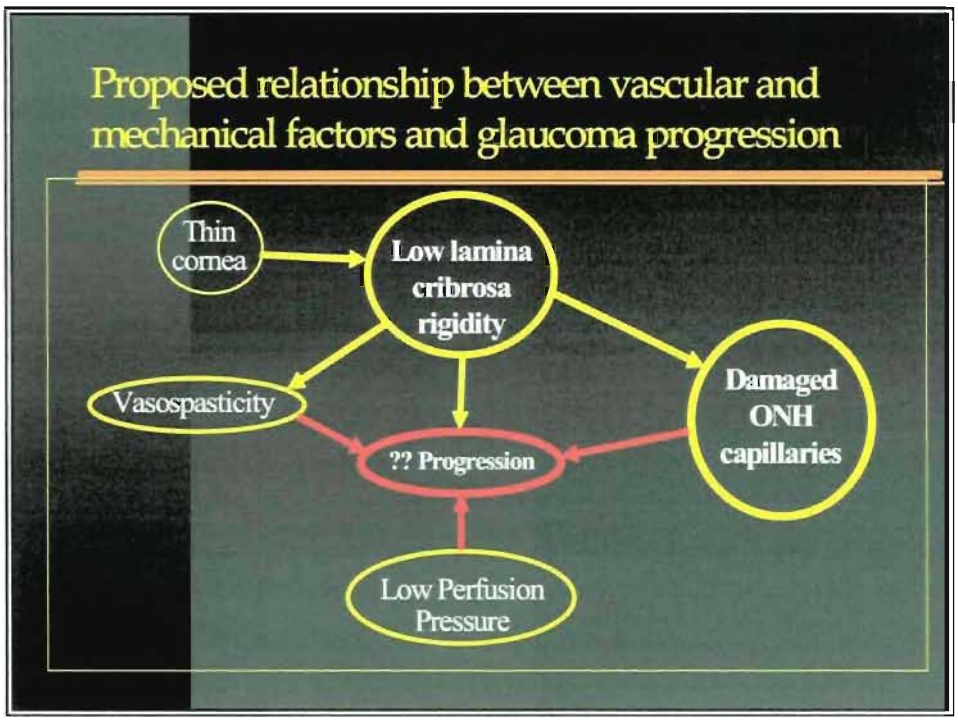

Figure 10.1: Proposed relationship between vascular and mechanical factors and the progression of glaucomatous optic neuropathy.

Our model agrees with the established fact that IOP is a significant risk factor for the development or progression of glaucomatous optic neuropathy but also emphasizes the role of other factors as reduced ONH blood flow, defective ONH autoregulation, peripheral vasospasm and CCT. We also introduce the hypothesis that the structural compliance of the lamina cribrosa could be a useful indicator for the development of early glaucoma (Figure 10.1).

The results also represent possible means for assessment of glaucoma patients with higher risk for progression and visual field loss. Patients with greater lamina cribrosa compliance may be identified and more aggressive therapy could be initiated accordingly.

The current studies implement a set of clinical investigations that could significantly improve our understanding of the pathogenesis of glaucoma. Such studies may, furthermore, allow us to determine patient-specific criteria or endpoints for normalization of ocular pathophysiology and stabilization of visual field. The strategy used combines both perfusion changes as well as morphologic responses to sustained therapeutic IOP reduction. The studies might also expand our understanding of the role of autoregulation, vasospasm and ONH biomechanics in glaucoma and could therefore contribute to the development of new therapeutic targets for that disease.

This data, while far from being conclusive, suggests that there may be interplay between the factors evaluated in these studies and disease progression. The data also underscores the proposed links between vasospasm, optic nerve blood flow and ONH compliance on the one hand, and the pathogenesis of glaucomatous optic neuropathy on the other. A large scale prospective study might lead to a clearer understanding of such factors and the way we view the pathophysiology, risk assessment, and treatment of glaucoma.

APPENDICES

APPENDIX I: PRESENTATION OF RESULTS

A. PUBLISHED ARTICLES

1. Lesk MR, **Hafez AS**, Descovich D. Relationship between central corneal thickness and changes of optic nerve head topography and blood flow following IOP reduction in open angle glaucoma and ocular hypertension. Arch Ophthalmol. 2006, 124:1568-1572.
2. **Hafez AS**, Lesk MR. Correlation between finger blood flow and changes in ocular blood flow following therapeutic intraocular pressure reduction in glaucoma patients and ocular hypertensives. J of Glaucoma 2005, 14: 448-454.
3. **Hafez AS**, Bizzarro RLG, Lesk MR. Optic nerve head blood flow in glaucoma patients, ocular Hypertensives and normal subjects measured by scanning laser Doppler flowmetry. Am J Ophthalmol 2003, 136(6): 1022-1031.
4. **Hafez AS**, Bizzarro RLG, Rivard M, Trabut I, Lovasik JV, Kergoat H, Lesk MR. Reproducibility of retinal and optic nerve head perfusion measurements using scanning laser Doppler flowmetry. Ophthalmic Surg Lasers Imaging 2003; 34 (5): 422-32.
5. **Hafez AS**, Bizzarro RLG, Rivard M, Lesk MR. Changes in optic nerve head blood flow after therapeutic intraocular pressure reduction in glaucoma patients and ocular hypertensives. Ophthalmology 2003, 110(1): 201-210.

B. SUBMITTED ARTICLES

1. **Hafez AS,** Bizzarro RLG, Lesk MR. Changes in optic nerve head topography following therapeutic intraocular pressure reduction in glaucoma patients and ocular hypertensives. Submitted, Invest Ophthalmol Vis Sci 2007.
2. **Hafez AS**., Papamatheakis D, Descovich D, Lesk MR. Relatonship between glaucomatous visual field progression, vasospasticity, and changes in optic nerve head topography and blood flow at the time of initial IOP reduction: A prospective pilot study. Submitted, Am J Ophthalmol 2007.

C. PUBLISHED ABSTRACTS

1. Lesk MR, Papamatheakis D, Descovich D, **Hafez AS**. Relatonship between glaucomatous visual field progression, vasospasticity, and changes in optic nerve head topography and blood flow at the time of initial IOP reduction: A prospective pilot study. Invest Ophthalmol Vis Sci 2005, 46: E-Abstract 4782.
2. Lesk MR, **Hafez AS**, Descovich D. Relationship between central corneal thickness, changes in optic nerve head topography and blood flow following IOP change in open angle glaucoma and ocular hypertension. Invest Ophthalmol Vis Sci 2004, 45: E-Abstract 4439.
3. **Hafez AS**, Bizzarro RLG, Lesk MR. Correlation between neuroretinal rim blood flow measured with scanning laser Doppler flowmetry and clinical cup/disc ratio in ocular hypertension. Invest Ophthalmol Vis Sci 2003, 44: E-Abstract 127.

4. **Hafez AS,** Bizzarro RLG, Lesk MR. Correlation between finger blood flow and changes in ocular blood flow following therapeutic intraocular pressure reduction in glaucoma patients and ocular hypertensives. Invest Ophthalmol Vis Sci 2002, 43: E-Abstract 313.

5. Bizzarro RLG, **Hafez AS**, Lesk MR. Changes in optic nerve head blood flow after therapeutic intraocular pressure reduction in primary open angle glaucoma and juvenile open angle glaucoma patients. Invest Ophthalmol Vis Sci 2002, 43: E-Abstract 314.

6. Harasymowycz PJ, **Hafez AS**, Lesk MR. The validation of importing the disc area contour line in Heidelberg Retina Tomography: Do disc area measurements change following IOP reduction? Invest Ophthalmol Vis Sci 2002, 43: E-Abstract 257.

7. **Hafez AS**, Bizzarro RLG, Rivard M, Lesk MR. Changes in optic nerve head blood flow after therapeutic intraocular pressure reduction in glaucoma patients. Invest Ophthalmol Vis Sci 2001; 42(4): S20.

8. Lesk MR, **Hafez AS,** Bizzarro RLG. Changes in optic nerve head topography following therapeutic intraocular pressure reduction in glaucoma patients and ocular hypertensives. Invest Ophthalmol Vis Sci 2001; 42(4): S132.

9. Bizzarro RLG, **Hafez AS**, Lesk MR. Optic nerve head blood flow in glaucoma patients, ocular hypertensives and normal subjects measured by scanning laser Doppler flowmetry. Invest Ophthalmol Vis Sci 2001; 42(4): S21.

10. Bizzarro RLG, **Hafez AS**, Rivard M, Trabut I, Lesk MR. Reproducibility of retinal and optic nerve head perfusion measurements using scanning laser Doppler flowmetry. Invest Ophthalmol Vis Sci 2000; 41(4): S556.
11. Lesk MR, **Hafez AS,** Bizzarro RLG, Descovich D. Correlation between finger blood flow and changes in optic nerve head blood flow following therapeutic intraocular pressure reduction in glaucoma patients and ocular hypertensives. ISIE 2003.
12. **Hafez AS,** Bizzarro RLG, Lesk MR. Reduced neuroretinal rim blood flow as a risk factor for the development of glaucomatous optic neuropathy in ocular hypertensives. Can J Ophthalmol 2003; 37(2): 94.
13. **Hafez AS,** Bizzarro RLG, Lesk MR. Does finger blood flow correlate with changes in optic nerve head blood flow following therapy for open-angle glaucoma and ocular hypertension? Can J Ophthalmol 2002; 37(2): 94.
14. **Hafez AS**, Bizzarro RLG, Rivard M, Lesk MR. Scanning laser Doppler flowmetry: A method to evaluate blood flow changes in glaucoma. Can J Ophthalmol 2000; 35(2): 99.

D. ORAL PRESENTATIONS

1. Relation between glaucoma progression and changes in optic nerve head topography and blood flow at the time of initial reduction of intraocular pressure

reduction. A prospective pilot study. Canadian Ophthalmological Society Annual Meeting. Edmonton, AB, June 2005.

2. Reduced neuroretinal rim blood flow as a risk factor for the development of glaucomatous optic neuropathy in ocular hypertensives. Canadian Ophthalmological Society Annual Meeting. Halifax, NS, June 2003.
3. Does finger blood flow correlate with changes in optic nerve head blood flow following therapy for open-angle glaucoma and ocular hypertension? Canadian Ophthalmological Society Annual Meeting. Hull, QC, June 2002.
4. Changes in Optic Nerve Head Topography Following Therapeutic Intraocular Pressure Reduction in Glaucoma Patients and Ocular Hypertensives. Canadian Ophthalmological Society Annual Meeting. Toronto, ON, June 2001.
5. Scanning laser Doppler flowmetry. A Method to Evaluate Changes in Optic Nerve Head Blood Flow in Glaucoma Patients. Canadian Ophthalmological Society Annual Meeting. Whistler, BC, June 2000.

Reproducibility of Retinal and Optic Nerve Head Perfusion Measurements Using Scanning Laser Doppler Flowmetry

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■ **BACKGROUND AND OBJECTIVE:** To evaluate the reproducibility of full-field perfusion analysis using scanning laser Doppler flowmetry (SLDF) for perfusion measurements of the neuroretinal rim of the optic nerve head and the peripapillary retina in patients with open-angle glaucoma or ocular hypertension, and in normal subjects. **AQ1**

■ **PATIENTS AND METHODS:** Perfusion measurements of the neuroretinal rim and the peripapillary retina were performed on 20 patients with open-angle glaucoma or ocular hypertension (group G) and 20 normal volunteers (group N), using scanning laser Doppler flowmetry.**AQ1** Each subject underwent two independent sessions, 30 minutes apart, each involving 5 high quality images. Intrasession and intersession reproducibility coefficients for flow, volume, and velocity were calculated for a single image and for means of 3 and 5 images using analysis of variance models.

■ **RESULTS:** Using a mean of 5 measurements for flow, the intrasession coefficient of reliability was 0.99 each for the rim, nasal retina, and temporal retina in group G and 0.93, 0.93, and 0.95, respectively, in group N. The intersession coefficient of reliability for flow was 0.99 for the rim, 0.95 for the nasal retina, and 0.87 for the temporal retina in group G and 0.87, 0.82, and 0.80, respectively, in group N. Compared with single image analysis, intrasession and intersession reproducibility were generally better when a mean of 3 images and substantially better when a mean of 5 images was used.

■ **CONCLUSION:** SLDF full-field perfusion analysis is markedly more reproducible than the original software using 10 × 10 pixels windows.**AQ1** Obtaining mean values for at least 3 images improves the intrasession and intersession reproducibility of this technique.

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INTRODUCTION

Substantial evidence suggests that defective perfusion of the optic nerve head and peripapillary retina contributes to the development and progression of glaucomatous optic neuropathy in many patients.[1-4] Abnormalities of ocular blood flow in glaucoma have been shown using many techniques, including fluorescein angiography,[5] color Doppler imaging,[6,7] laser Doppler flowmetry,[8] and pulsatile ocular blood flow.[9] The ability to accurately determine the perfusion of

the optic nerve head is fundamental to furthering our understanding of the pathogenesis of optic nerve head damage in glaucoma.[10,11]

In 1992, Riva et al.[12] introduced single-point laser Doppler flowmetry to obtain rapid and noninvasive hemodynamic measurements in the optic nerve head. Since then, several studies have reported measurements of blood flow with this method in the optic nerve head,[13,14] the retina,[15,16] and the choroid.[17,18] The technique has been shown to have good reproducibility. It is thought to measure blood flow in the superficial layers of the disc and not in the deeper layers of the optic nerve head.[19,20]

In 1995, Michelson and Schmauss[21] described the scanning laser Doppler flowmeter, a noninvasive device that combines the laser Doppler flowmeter with a scanning laser system. It allows the visualization of perfused vessels in a scan area of 2.7 × 0.7 mm and the quantification of perfusion parameters in discrete locations of the optic nerve head and peripapillary retina. In an experimental setup, scanning laser Doppler flowmetry (SLDF) was shown to be both valid and reproducible. Michelson et al.[22] were able to estimate the capability of SLDF to measure the velocity of a moving plane in absolute units. Chauhan and Smith[23] conducted a series of experiments using fluids driven over a range of pump flow rates into glass capillaries and reported a linear relationship between flow measured by SLDF and actual flow within a given operating range.

When first introduced, SLDF software permitted analysis by placement of a measurement window of variable size (1 × 1, 4 × 4, or 10 × 10 to 50 × 50 pixels) in the region of interest within the scan area. Using this software, reproducibility of the neuroretinal rim blood flow was considered poor, with an intersession coefficient of reliability of 0.36 in patients with glaucoma and 0.47 in normal volunteers,[24] although peripapillary retinal blood flow appeared to be more reproducible.[22,24,25] Investigators attributed the large variation in rim blood flow to focusing difficulties on the neuroretinal rim due to its low reflectivity.[24] Such an effect can reduce the power to detect statistical differences between various groups of patients or following experimental manipulation of optic nerve head blood flow.

In 1998, Michelson et al.[26] described a new method for automatic full-field perfusion image analysis. They developed an algorithm to improve SLDF measurements in the optic nerve head and peripapillary retina by reducing the influence of heterogeneity of the perfusion map and of pulsations associated with the heart beat. As opposed to conventional analyses of SLDF images by a measurement window of 10 × 10 pixels whose position is selected by the operator, this technique calculates the perfusion parameters of all valid pixels in the entire scan area and computations are then performed using the perfusion values retained for analysis. The resulting perfusion map is subdivided into temporal and nasal peripapillary retinal areas, as well as neuroretinal rim area.

Michelson et al.[26] reported intersession coefficients of reliability for the flow parameter in the nasal and temporal peripapillary retina of 0.76 and 0.73, respectively, in normal subjects. This intersession reliability was in the same range as the reliability of the conventional analysis using measurement windows.

In a recent study, Iester et al.[27] reported an increased variability in the measurements of the neuroretinal rim compared with the peripapillary retina with automatic full-field perfusion image analysis. The authors reported an intra-image (the same image analyzed 5 times by the same observer) coefficient of variation of 20.3% (range, 0.5% to 28%) and an inter-image (3 consecutive images analyzed once by the same observer) coefficient of variation of 24.4% (range, 2.0% to 30%). However, no reproducibility data have been reported using this technique when perfusion values were obtained from the mean of the multiple perfusion images.

The purpose of this study was to evaluate the intrasession and intersession reproducibility using the SLDF full-field perfusion analysis of the neuroretinal rim and peripapillary retina in our population of patients with open-angle glaucoma or ocular hypertension and normal volunteers. We also examined the reproducibility when data were derived from multiple successive perfusion images in an attempt to determine the optimal number of measurements needed to obtain highly reproducible perfusion values.

PATIENTS AND METHODS

The study was approved by the research committee of Maisonneuve-Rosemont Hospital, University of Montreal. Informed consent was obtained from each subject prior to enrollment.

Thirty-two patients with open-angle glaucoma or ocular hypertension were recruited from the glaucoma clinic of the hospital and assigned to group G. Twenty-nine normal subjects were also recruited, mostly from

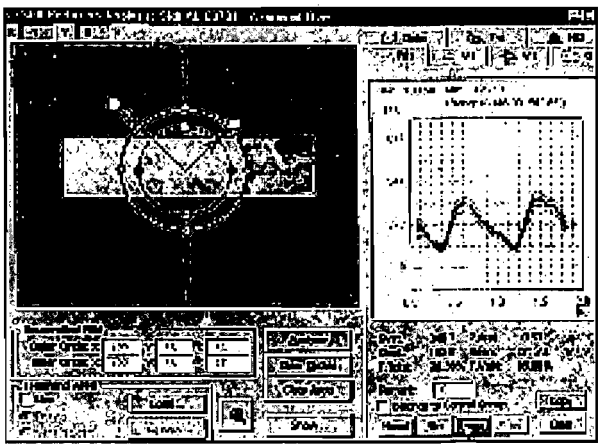

Figure. AQ5

friends and spouses of patients with glaucoma and volunteers, and assigned to group N.

Patients with open-angle glaucoma had gonioscopically open angles and fulfilled two of the following three criteria: a history of intraocular pressure (IOP) greater than 21 mm Hg, characteristic nerve fiber bundle visual field defects, and glaucomatous optic neuropathy. Patients with ocular hypertension had a history of repeated IOP measurements greater than 24 mm Hg, normal visual fields, and normal optic nerve head appearance. Normal subjects had an IOP below 21 mm Hg, normal visual fields, normal optic nerve head appearance, and no family history of glaucoma.

Subjects were excluded from the study if they had other abnormal ocular findings (apart from pseudophakia), if significant media opacities precluding SLDF imaging were present, or if at any time they were unable to cooperate.

During the prestudy visit of each patient, medical and ocular history were taken. Best-corrected visual acuities, IOPs, and refractive errors were measured. A routine ophthalmologic examination including biomicroscopy, gonioscopy, and ophthalmoscopy was performed and recent automated perimetry (Humphrey Field Analyzer, program 24-2; Humphrey Instruments, San Leandro, CA) was used to evaluate the visual field. Blood pressure and heart rate were recorded. Ocular perfusion pressure (OPP) was calculated according to the formula: OPP = 2/3 (diastolic blood pressure + 1/3 [systolic blood pressure – diastolic blood pressure]) – IOP.[28]

The scanning laser Doppler flowmeter used in this study (Heidelberg Retina Flowmeter [HRF]; Heidelberg Engineering GmbH, Heidelberg, Germany) is a noninvasive instrument combining a laser Doppler flowmeter with a scanning laser technique. It measures the amount of backscattered light at different locations in the tissue of interest within a short period of time. An infrared diode laser with a wavelength of 780 nm is used. The area examined measures 2.7 × 0.7 mm and is composed of 64 horizontal lines, each with 256 points giving an approximate spatial resolution of 10 μm. Each line is scanned sequentially a total of 128 times with a total acquisition time of 2.05 seconds. A discrete fast Fourier transformation is performed over the 128 intensity values for each retinal point, generating a spectrum of the Doppler shift for each point. A two-dimensional map of microvascular perfusion of the area to be studied is thus obtained.

The automatic full-field perfusion image analysis software (SLDF, version 3.3)AQ2 (Figure) developed by Michelson et al.[26] enhances the computations previously generated by the HRF through (1) elimination of all pixels with incorrect brightness (overexposed or underexposed) and pixels of cup area, as well as marking of eye movements (saccades), that lead to erroneous perfusion data; (2) exclusion of retinal vessels with a diameter greater than 30 μm, whose flow velocities are too high for the sampling frequency afforded by this technique; (3) an analysis based on the average of all valid pixels in the scan area rather than data obtained from a discrete target square; (4) a perfusion map that can be subdivided into three critical regions of interest for analysis (the temporal and nasal peripapillary retinal areas and the rim area) and each analyzed separately; (5) a heart beat–associated pulsation of capillary blood flow estimated by plotting the mean flow of each horizontal line against time; and (6) the ability to save and retrieve all data and settings (position of the rim circles and calculation parameters) used in an analysis for subsequent follow-up or reanalysis.

Full-field perfusion analysis of HRF images is thus obtained, from which numerical readings of "flow" (distance traveled by all moving red blood cells per unit of time), "volume" (number of moving red blood cells), and "velocity" (mean red blood cell speed) are given in arbitrary units.

For testing, pupils 3 mm in diameter or smaller were dilated. The subject was asked to use the fellow eye for fixation on a target and to refrain from movement and blinking during image acquisition. The fundus camera was adjusted until a well-focused, evenly

illuminated and centered view of the optic nerve head was obtained. A total of 7 to 9 images were then acquired sequentially at each of two independent sessions, 30 minutes apart, focusing on the superficial retina. To obtain a consistent and optimum exposure within or between sessions, illumination intensity was adjusted to the maximum level that did not result in overexposed pixels on the peripapillary retina. Also, the angulation of the fundus camera and its distance from the eye was kept constant throughout the process of image acquisition.

All images were reviewed by the same observer and the best 5 images from each session in terms of focusing, centration, brightness, and absence of movements were chosen prior to data analysis. Subjects with images of poor quality were excluded from the study. Each of the 5 chosen images was then analyzed for measurements of blood flow, volume, and velocity for the nasal and temporal peripapillary retinal areas and the neuroretinal rim area.

Statistical evaluations were then performed on both groups for flow, volume, and velocity using analysis of variance (ANOVA). The reproducibility of the measurements was assessed by estimating intraclass correlation coefficients according to two random effect ANOVA models. The intraclass correlation coefficient is considered to be the standard statistical indicator of reproducibility based on the classic test theory developed by Spearman.[29] It is defined as $\sigma_T^2/(\sigma_T^2 + \sigma_e^2)$, where σ_T^2 is the component of "true" variance (variance between subjects) and σ_e^2 is the component of variance reflecting measurement error (variance within subjects or "noise"). If the noise is small relative to the true variation, the value of the intraclass correlation coefficient will be closer to 1.0, thus reflecting a high level of reproducibility. Similarly, an intraclass correlation coefficient of 0.5 indicates that only half of all variability is due to real differences between subjects and the other half is due to noise.

Intrasession Reproducibility

The data from each session were analyzed by adjusting one-way random effect ANOVA models and calculating three intraclass correlation coefficients. R1 is the intraclass correlation coefficient for a single measurement (image). R3 and R5 are obtained by applying the Spearman–Brown formula (introduced by Lord and Novik).[30] They correspond to stepped-up intraclass correlation coefficients for the mean of 3 and 5 measurements (images), respectively. In addition, within-subject variation was studied by calculating for each subject the coefficient of variation for the mean of 5 measurements from the first session. Mean (± standard deviation [SD]) coefficients of variation were then calculated for both groups.

Intersession Reproducibility

Mixed two-way ANOVA models were adjusted to estimate intraclass correlation coefficients R1, R3, and R5. For R1 and R3, images were selected at random from the set of 5 images available for each session. Changes between sessions for the mean of 5 measurements were also examined by computing for each subject the percent change in mean values relative to the mean value from the first session. Mean (± SD) percent changes were then calculated for both groups. Because only two sessions were performed, mean percent changes rather than coefficients of variation were calculated to estimate the intersession variability.

The precision of the estimated coefficients of reliability was then ascertained by computing 95% confidence intervals. Confidence intervals were used to assess whether differences between coefficients of reliability for a single image, a mean of 3 images, or a mean of 5 images were statistically significant by examining whether their confidence intervals overlap. Confidence intervals were also used to assess a statistically significant difference in coefficients of reliability between the two study groups.

RESULTS

SLDF measurements were performed on 32 patients with open-angle glaucoma or ocular hypertension and 29 normal subjects. Good quality images were obtained from 24 patients with open-angle glaucoma or ocular hypertension and 21 normal subjects and were analyzed. Of these, 4 patients with open-angle glaucoma and 1 normal subject were excluded for invalid rim data. Thus, data are presented for 20 patients with open-angle glaucoma or ocular hypertension (group G) and 20 normal volunteers (group N).

Causes of poor quality images were primarily excessive eye movements and media opacities in the cornea, lens, or vitreous. Invalid rim data was the term generated by the software in cases where the extremely low reflectivity of the optic nerve head usually accompanied by high cup-to-disc ratio caused an inadequate number of pixels in the neuroretinal rim tissue

TABLE 1
Intraocular Pressure, Cup-to-Disc Ratio, and Ocular Perfusion Pressure in Group G and Group N

Parameter	Group G (N = 20) Mean	Group G (N = 20) SD	Group N (N = 20) Mean	Group N (N = 20) SD	P*
Age, y	65.9	12.4	63.6	9.8	.460
Intraocular pressure	22.6	5.63	16.9	2.65	.001
Cup-to-disc ratio	0.6	0.23	0.2	0.12	.000
Ocular perfusion pressure	44.29	6.60	48.19	7.18	.063

Group G = patients with open-angle glaucoma or ocular hypertension; Group N = normal subjects; SD = standard deviation.
**Two-tailed Student's t test.*

to be available for analysis. Of the 4 patients with open-angle glaucoma excluded for invalid rim data; 3 had a cup-to-disc ratio of greater than 0.8. The patient with glaucoma who had a cup-to-disc ratio of less than 0.8 and the normal subject who had a cup-to-disc ratio of 0.3 had an unusually low reflectivity of the neuroretinal rim. All 5 subjects were automatically excluded by the SLDF software.

The mean (± SD) age was 65.9 ± 12.4 years (range, 42 to 80 years) for group G and 63.6 ± 9.8 years (range, 41 to 77 years) for group N. No statistically significant difference in age was detected between groups (P = 0.460, two-tailed Student's t test). There were 10 men (50%) and 10 women (50%) in group G, and 9 men (45%) and 11 women (55%) in group N. Group G included 16 patients (80%) with open-angle glaucoma and 4 patients (20%) with ocular hypertension.

Six subjects (30%) in group G and 4 subjects (20%) in group N had medically controlled systemic hypertension. Three subjects (15%) in group G and 2 subjects (10%) in group N had diabetes mellitus.

Patients with open-angle glaucoma or ocular hypertension within group G were heterogeneous regarding the control of their disease. Nineteen of the 20 subjects in this group were receiving topical hypotensive therapy. Of the 20 subjects in group G, 17 (85%) were considered to have uncontrolled IOP and 3 (15%) had adequately controlled IOP, as evaluated by their IOP, optic nerve head appearance, and visual field. The average (± SD) mean defect on automated perimetry in this group was -7.05 (± 7.84), with a range of +0.26 to -27.9. One subject in group G (5%) and 3 subjects (15%) in group N had previous cataract surgery in the study eye, 1 subject in group G (5%) had previous glaucoma surgery, and 1 subject (5%)**AQ3** had previous combined cataract and glaucoma surgery.

The means (± SD) of age, IOP, clinical cup-to-disc ratio, and ocular perfusion pressure of the subjects in both study groups are shown in Table 1. Statistically significant differences between group G and group N were demonstrated in two of the four parameters ($P \leq .001$, two-tailed Student's t test). Marginally insignificant differences were reported between the two study groups in the means of ocular perfusion pressure (P = .08).

Intrasession Reproducibility

In group G, the coefficient of reliability of a single image for flow for each region (rim, nasal retina, and temporal retina) was 0.94. Coefficients of reliability for flow were 0.98 using a mean of 3 images and 0.99 using a mean of 5 images, for each region (Table 2).

In group N, the coefficient of reliability for a single image for flow was 0.54 for the rim, 0.67 for the nasal peripapillary retina, and 0.80 for the temporal peripapillary retina. However, the coefficient of reliability for flow improved to 0.78 to 0.92 for a mean of 3 images and 0.93 to 0.95 for a mean of 5 images, depending on location (Table 3). Overall, the intrasession coefficient of reliability of 5 images for the three areas tested was higher in group G than in group N (flow: 0.99 vs 0.93 to 0.95; volume: 0.97 to 0.99 vs 0.94 to 0.97; velocity: 0.98 to 0.99 vs 0.88 to 0.96, respectively). In many locations, this difference was statistically significant, as indicated by the non-overlap of the 95% confidence intervals.

Intrasession variability of flow as measured by mean coefficients of variation for 5 perfusion images revealed 9.5% to 19.8% variability in group G compared with 11.4% to 16.4% variability in group N, depending on location (Table 4).

TABLE 2

Intrasession Coefficients of Reliability (R) and 95% Confidence Intervals (CI_{95}) for Group G (N = 20) as Calculated for One Image (R1), a Mean of Three Images (R3), and a Mean of Five Images (R5)

Parameter	Perfusion Values		Coefficient of Reliability		
	Mean	SD	R1 (CI_{95})	R3 (CI_{95})	R5 (CI_{95})
Volume					
Nasal	20.62	7.26	0.93 (0.8–0.97)	0.97 (0.95–0.99)	0.99 (0.98–0.99)
Rim	17.43	7.21	0.86 (0.74–0.94)	0.95 (0.89–0.98)	0.97 (0.95–0.99)
Temporal	21.75	9.49	0.93 (0.85–0.97)	0.97 (0.95–0.99)	0.98 (0.97–0.99)
Flow					
Nasal	351.0	165.5	0.94 (0.88–0.97)	0.98 (0.96–0.99)	0.99 (0.98–1.00)
Rim	256.6	195.6	0.94 (0.88–0.97)	0.98 (0.95–0.99)	0.99 (0.98–1.00)
Temporal	337.5	129.0	0.94 (0.87–0.97)	0.98 (0.95–0.99)	0.99 (0.97–0.99)
Velocity					
Nasal	1.27	0.53	0.95 (0.89–0.98)	0.98 (0.96–0.99)	0.99 (0.98–1.00)
Rim	1.20	0.71	0.92 (0.84–0.96)	0.97 (0.94–0.99)	0.98 (0.96–0.99)
Temporal	1.20	0.42	0.95 (0.91–0.98)	0.98 (0.97–0.99)	0.99 (0.97–0.99)

Group G = patients with open-angle glaucoma or ocular hypertension; SD = standard deviation.

TABLE 3

Intrasession Coefficients of Reliability (R) and 95% Confidence Intervals (CI_{95}) for Group N (N = 20) as Calculated for One Image (R1), a Mean of Three Images (R3), and a Mean of Five Images (R5)

Parameter	Perfusion Values		Coefficient of Reliability		
	Mean	SD	R1 (CI_{95})	R3 (CI_{95})	R5 (CI_{95})
Volume					
Nasal	18.09	3.98	0.76 (0.57–0.89)	0.90 (0.80–0.96)	0.94 (0.89–0.96)
Rim	17.99	4.33	0.84 (0.69–0.92)	0.94 (0.87–0.97)	0.95 (0.91–0.99)
Temporal	21.44	5.91	0.85 (0.72–0.93)	0.95 (0.89–0.98)	0.97 (0.94–0.99)
Flow					
Nasal	279.1	79.9	0.67 (0.44–0.84)	0.86 (0.70–0.94)	0.93 (0.86–0.97)
Rim	272.0	92.9	0.54 (0.28–0.76)	0.78 (0.54–0.91)	0.93 (0.87–0.97)
Temporal	307.5	85.5	0.80 (0.63–0.91)	0.92 (0.84–0.97)	0.95 (0.91–0.98)
Velocity					
Nasal	1.04	0.27	0.69 (0.47–0.85)	0.87 (0.72–0.94)	0.93 (0.87–0.97)
Rim	1.17	0.28	0.61 (0.36–0.80)	0.82 (0.63–0.92)	0.88 (0.78–0.95)
Temporal	1.10	0.29	0.76 (0.58–0.89)	0.91 (0.80–0.96)	0.96 (0.92–0.98)

Group N = normal subjects; SD = standard deviation.

Higher intrasession mean coefficient of variation for the rim than for the peripapillary retina was present in all parameters in both study groups. The variability was not uniform in either group, with some subjects

TABLE 4
Intrasession Variability of Five Images as Measured by Mean (± SD) Coefficients of Variation According to Parameter, Location of Measurement, and Study Group

Parameter	Mean (± SD) Coefficients of Variation	
	Group G (N = 20)	Group N (N = 20)
Volume		
Nasal	7.7 ± 4.1	8.8 ± 6.0
Rim	14.4 ± 8.7	10.0 ± 5.5
Temporal	7.8 ± 3.6	9.4 ± 4.6
Flow		
Nasal	9.5 ± 5.3	11.4 ± 7.7
Rim	19.8 ± 15.9	16.4 ± 12.3
Temporal	10.3 ± 5.1	11.6 ± 5.4
Velocity		
Nasal	9.6 ± 5.7	11.2 ± 7.0
Rim	22.9 ± 16.8	13.8 ± 10.1
Temporal	9.0 ± 4.0	11.5 ± 4.6

SD = standard deviation; Group G = patients with open-angle glaucoma or ocular hypertension; Group N = normal subjects.

demonstrating small variations in measurements and others showing large variations (data not shown).

Intersession Reproducibility

In group G, the intersession coefficient of reliability of a single image for flow was 0.99 for the rim, 0.95 for the nasal retina retina, and 0.73 for the temporal retina (Table 5). The coefficient of reliability for virtually all parameters was higher when a mean of 3 or 5 images was used, although not significantly so.

In group N, the coefficient of reliability of a single image for the parameter flow was 0.45 for the rim, 0.75 for the nasal, and 0.42 for the temporal peripapillary retina (Table 6). However, the coefficient of reliability improved to 0.84 to 0.87 for a mean of 3 images and 0.82**AQ4** to 0.87 for a mean of 5 images, depending on location. Volume and velocity parameters showed similar patterns of improved reliability when multiple images were used for analysis (Table 6).

Intersession variability of the parameter flow as measured by mean percent change for 5 perfusion images revealed 9.5% to 10.6% variability in group G compared with 9.8% to 12.5% variability in group N, depending on location (Table 7).

In both study groups, the intersession mean percent change for the rim was generally comparable to that of the peripapillary retina. Again, the variability was not uniform among subjects in either group, with some subjects demonstrating small variations in measurements and others demonstrating large variations (data not shown).

DISCUSSION

SLDF represents a significant advance in the noninvasive evaluation of the microvascular hemodynamics of the optic nerve head and the peripapillary retina. However, it is important to determine its reproducibility, validity, and limitations before its application to a clinical setting.

Our study was conducted with 61 subjects recruited from the glaucoma clinic of the hospital. However, we were unable to obtain good quality images for 16 of the 61 subjects and 5 were excluded for invalid rim data as determined by the software. We attempted to recruit all subjects having clear media and good fixation, usually assessed by ability to adequately perform automated perimetry. However, we were unable to produce good quality images in one-third of those initially considered as good candidates. This observation points to the applicability of the technique in clinical practice. However, for those subjects in whom the technique can be successfully applied, reproducibility was generally high.

Using the 10 × 10 pixel measurement box placed in a retinal location in normal subjects, other investigators have reported an intrasession coefficient of reliability for flow of 0.84[21] and an intersession coefficient of reliability ranging from 0.62 to 0.82.[22] However, with placement of the box in a rim location, the intersession coefficient of reliability for flow dropped to 0.47 in normal volunteers and 0.36 in patients with glaucoma.[24] Using the same technique, reported intrasession coefficients of variation for flow in a retinal location of normal subjects ranged between 6.6%[26] and 12%[25] and intersession coefficients of variation ranged between 14%[25] and 22%.[24] In a rim location, the reported intersession coefficient of variation was 25%.[24] In one study that used flow histograms and pixel-by-pixel analysis of the entire perfusion image, the intersession coefficient of varia-

TABLE 5

Intersession Coefficients of Reliability (R) and 95% Confidence Intervals (CI_{95}) for Group G (N = 20) as Calculated for One Image (R1), a Mean of Three Images (R3), and a Mean of Five Images (R5)

Parameter	Coefficient of Reliability		
	R1 (CI_{95})	R3 (CI_{95})	R5 (CI_{95})
Volume			
Nasal	0.91 (0.80–0.97)	0.93 (0.84–0.97)	0.95 (0.88–0.98)
Rim	0.90 (0.76–0.96)	0.92 (0.81–0.97)	0.95 (0.88–0.98)
Temporal	0.84 (0.63–0.95)	0.92 (0.80–0.97)	0.93 (0.83–0.97)
Flow			
Nasal	0.95 (0.88–0.98)	0.96 (0.89–0.98)	0.95 (0.89–0.98)
Rim	0.99 (0.98–1.00)	0.98 (0.95–0.99)	0.99 (0.97–1.00)
Temporal	0.73 (0.44–0.89)	0.86 (0.68–0.94)	0.87 (0.71–0.95)
Velocity			
Nasal	0.94 (0.86–0.98)	0.95 (0.88–0.98)	0.96 (0.89–0.98)
Rim	0.85 (0.66–0.94)	0.95 (0.87–0.98)	0.96 (0.90–0.98)
Temporal	0.78 (0.53–0.91)	0.89 (0.75–0.96)	0.89 (0.75–0.96)

Group G = patients with open-angle glaucoma or ocular hypertension.

TABLE 6

Intersession Coefficients of Reliability (R) and 95% Confidence Intervals (CI_{95}) for Group N (N = 20) as Calculated for One Image (R1), a Mean of Three Images (R3), and a Mean of Five Images (R5)

Parameter	Coefficient of Reliability		
	R1 (CI_{95})	R3 (CI_{95})	R5 (CI_{95})
Volume			
Nasal	0.85 (0.67–0.94)	0.88 (0.71–0.95)	0.85 (0.65–0.94)
Rim	0.54 (0.14–0.79)	0.86 (0.68–0.94)	0.82 (0.59–0.92)
Temporal	0.80 (0.57–0.92)	0.93 (0.83–0.97)	0.92 (0.81–0.97)
Flow			
Nasal	0.75 (0.47–0.89)	0.87 (0.69–0.94)	0.82 (0.59–0.92)
Rim	0.45 (0.03–0.74)	0.87 (0.69–0.94)	0.87 (0.68–0.95)
Temporal	0.42 (0.00–0.72)	0.84 (0.64–0.93)	0.80 (0.56–0.92)
Velocity			
Nasal	0.78 (0.53–0.91)	0.88 (0.73–0.95)	0.83 (0.63–0.93)
Rim	0.28 (0.00–0.64)	0.79 (0.54–0.91)	0.67 (0.33–0.85)
Temporal	0.59 (0.21–0.82)	0.84 (0.65–0.93)	0.82 (0.61–0.93)

Group N = normal subjects.

tion for the parameter flow decreased from 30.1% to 16.3%.[31]

The technique of SLDF with the conventional evaluation of 10 × 10 pixel windows using the origi-

TABLE 7
Intersession Variability of the Mean (± SD) of Five Images as Measured by Mean Percent Change According to Parameter, Location of Measurement, and Study Group

Parameter	Mean (± SD) Percent Change	
	Group G (N = 20)	Group N (N = 20)
Volume		
Nasal	10.4 ± 6.4	9.1 ± 8.6
Rim	10.7 ± 8.6	11.0 ± 9.6
Temporal	7.4 ± 7.2	7.0 ± 7.6
Flow		
Nasal	10.6 ± 6.7	10.3 ± 13.3
Rim	9.7 ± 8.2	12.5 ± 12.0
Temporal	9.5 ± 9.4	9.8 ± 14.2
Velocity		
Nasal	9.7 ± 6.3	9.7 ± 11.5
Rim	14.4 ± 9.7	15.4 ± 17.7
Temporal	9.1 ± 8.8	8.8 ± 12.2

SD = standard deviation; Group G = patients with open-angle glaucoma or ocular hypertension; Group N = normal subjects.

nal HRF software has been reported to show the following limitations[19]: (1) artifacts caused by eye movements occurring during the 2 seconds of image acquisition disturb the Doppler shift signal; (2) subjective variation in the position of the measurement window selected by the operator between images; (3) placement of the 10 × 10 pixels measurement box on the neuroretinal rim of patients with glaucoma with a high cup-to-disc ratio and thin rim unavoidably includes an area of the cup or the peripapillary retina in the box, thus leading to erroneous data; (4) clinically insignificant media opacities degrade the quality of the images and increase the underlying noise and thus artifactually increase the overall perfusion values; and (5) monocular patients and patients with poor vision in the contralateral eye interfering with fixation cannot be tested.

The SLDF analysis software used in this study (originally developed by Michelson et al.[26]) is believed to enhance the computations previously generated by the HRF through elimination of the first three limitations. Using this full-field perfusion analysis, Michelson et al. reported an intersession coefficient of reliability of 0.74 for flow in the retina in normal subjects.[26] This intersession reliability was in the same range as the reliability of the conventional analysis of the 10 × 10 pixels measurement window used in the original HRF software. However, Michelson et al. used only one image, and consequently one measurement, per session for their computations.

The current study reports the first reproducibility data for SLDF full-field perfusion analysis when values were obtained from the means of multiple perfusion images and demonstrates that, using this method of analysis, reproducibility values are much higher than the reported values using the 10 × 10 pixels measurement window.

It is well established in the statistical literature that increasing the number of measurements is a good approach to improve reliability because the mean of several measurements is always more reliable than a single measurement.[29] Increasing the number of images (measurements) used in the analysis from 1 to 5 increased both intrasession (Tables 2 and 3) and intersession (Tables 5 and 6) reliability, and did so significantly for intrasession reliability. Improvements were still achieved with 3 images (measurements), although not to a level that was statistically significant.

In comparison to previous literature, this study also demonstrates improved intersession variability, whereas intrasession variability was similar to the previously reported range. It is believed that both the intrasession and the intersession variation contain elements due to technical variables or measurement errors, as well as elements due to true physiologic changes. In this study, all subjects were asked to sit back between the two sessions for an interval of 30 minutes in an attempt to maintain the same physiologic status throughout the imaging process, thus allowing us to examine primarily the variation due to technical variables. These variables might account for the individual differences in reproducibility that were observed in the different subjects.

Reproducibility was generally better in group G than in group N. However, this difference only reached statistical significance (as ascertained by the non-overlap of 95% confidence intervals) in the intrasession coefficient of reliability for the parameters of flow and velocity in the neuroretinal rim and the nasal peripapillary retina (Tables 2 and 3) and in the intersession coefficient of reliability for the parameter

flow in the neuroretinal rim (Tables 5 and 6). Better reproducibility in patients with glaucoma compared with normal subjects was similarly reported by Nicolela et al.[24] It might be explained in part by the fact that patients with glaucoma might have been better fixators as a result of previous training (ie, through repeated automated perimetry, optic disc photography, and multiple slit-lamp examinations). However, we could not detect a difference between either study group in terms of image quality.

A more likely explanation lies in the way in which the intraclass correlation coefficient (R) assesses reliability. As discussed previously, $R = \sigma_T^2/(\sigma_T^2 + \sigma_e^2)$, where σ_T^2 is the amount of variance between subjects (true variance) and σ_e^2 is the amount of variance within subjects (noise). In group N, σ_T^2 is relatively small because all subjects had normal blood flow. Group G, whose optic discs ranged from normal to advanced cupping, had a wider range of blood flow that produced a larger σ_T^2 value. Due to differences in σ_T^2 between the two groups, σ_e^2 could be the *same* in both groups and yet yield a *larger* intraclass correlation coefficient for group G. The perfusion values shown in Figures 2 and 3 demonstrate a larger between-subject variation in group G compared with group N, which explains the higher reliability estimates in that group.

Several authors have investigated the validity of SLDF for quantitative evaluation of retinal and optic nerve head perfusion. Michelson and Schmauss reported a significant linear relationship between SLDF flow and ocular perfusion pressure while varying the IOP by a suction cup in normal volunteers.[21] Furthermore, they compared measurements of corresponding retinal points by SLDF and a commercially available single-point laser Doppler flowmeter and reported a significant and linear relationship for flow, volume, and velocity in normal and glaucomatous eyes.[22] Other investigators[32,33] have shown that SLDF is appropriate for description of the effect of graded changes in blood gases on retinal hemodynamics. They noted that changes in measured blood flow at the optic nerve head occurred in the expected direction in response to blood gas perturbations.

SLDF analysis using HRF images permits noninvasive, high-resolution mapping of perfused vessels and capillaries of the optic nerve head and the peripapillary retina. However, the original software enables the quantification of blood flow, volume, and velocity only in selected areas of the perfusion map with poor reproducibility in the neuroretinal rim.[24] Full-field perfusion analysis is a newer approach that significantly reduces variables caused by the patient, operator, or device and permits highly reproducible quantification and documentation of the entire perfusion map, including the neuroretinal rim, in patients with glaucoma. Obtaining mean values from at least 3 images improves both the intrasession and the intersession reproducibility of this technique.

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Evaluation of Optic Nerve Head and Peripapillary Retinal Blood Flow in Glaucoma Patients, Ocular Hypertensives, and Normal Subjects

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• PURPOSE: To compare optic nerve head (ONH) and peripapillary retinal blood flow in subjects with open-angle glaucoma (OAG), ocular hypertension (OHT), and normal eyes (NOR) using full-field perfusion analysis of scanning laser Doppler flowmetry (SLDF) images.
• DESIGN: Prospective, nonrandomized clinical trial.
• METHODS: Twenty uncontrolled OAG patients, 20 uncontrolled OHT patients, and 20 normal volunteers were prospectively enrolled. Mean ONH and peripapillary retinal blood flow measurements were performed by SLDF version 3.3 using five Heidelberg Retina Flowmeter (Heidelberg Engineering, Heidelberg, Germany) images. Statistical evaluations were performed on the three study groups using one-way analysis of variance. Flow values of the neuroretinal rim of the ONH, nasal peripapillary retina, and temporal peripapillary retina were then correlated with the clinical parameters of age, cup/disk (C/D) ratio, intraocular pressure (IOP), visual field mean defect, maximum-recorded IOP, and ocular perfusion pressure.
• RESULTS: Neuroretinal rim blood flow in the OAG group was 158 ± 79 arbitrary units (au), whereas in the OHT group it was 277 ± 158 au, and in the NOR group it was 272 ± 93 au. Differences were statistically significant between the OAG group and each of the other groups ($P = .001$) but not between OHT and NOR groups ($P = .91$). Peripapillary retinal flow values showed no statistically significant differences between groups ($P = .76$ nasal and 0.93 temporal). Neuroretinal rim flow values showed a significant inverse correlation with C/D ratio ($P = .001$). Mean neuroretinal rim blood flow was significantly higher (350 ± 184 au) in the 10 OHT patients with C/D ratios < 0.4 when compared with the 10 OHT patients with larger C/D ratios (203 ± 79 au) ($P = .039$). Conversely, peripapillary retinal blood flow showed no significant correlation with any clinical parameter.
• CONCLUSION: Open-angle glaucoma patients had significantly lower blood flow in the ONH compared with OHT patients and normal volunteers. No significant differences in ONH blood flow were found between ocular hypertensives and normal volunteers. For peripapillary retinal blood flow, no significant difference was seen between any groups. Neuroretinal rim blood flow was significantly inversely correlated to increased C/D ratio. Ocular hypertensives with larger C/D ratios demonstrated significantly lower rim blood flow compared with those with smaller C/D ratios, suggesting that rim perfusion might be reduced in high-risk ocular hypertensives before the manifestation of visual field defects. (Am J Ophthalmol 2003;136:1022–1031. © 2003 by Elsevier Inc. All rights reserved.)

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IT IS WELL ESTABLISHED THAT ELEVATED INTRAOCULAR pressure (IOP) is an important risk factor in glaucoma. However, because elevated IOP alone is neither sufficient (in ocular hypertension [OHT]) nor necessary (in normotensive glaucoma [NTG]) for the development of glaucoma or its progression,[1–3] other causes of glaucoma have been investigated. Substantial evidence points to defective perfusion of the optic nerve head (ONH) as a

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risk factor for the development and progression of glaucomatous optic disk changes.[4,5]

Some of the main evidence implicating blood flow deficits in glaucoma is derived from fluorescein angiography. These studies[6–8] show delayed retinal circulation as well as impaired perfusion of the ONH, peripapillary retina, and choroid of glaucoma patients. The severity of perfusion defects progresses with the severity of glaucoma, and the defects correlate well with visual field loss and nerve fiber layer dropouts. Techniques using color Doppler imaging[9–13] and pulsatile ocular blood flow[14–17] demonstrated that both retrobulbar blood flow and bulk choroidal blood flow were reduced in glaucoma patients in comparison with normal subjects matched for age and circulatory risk factors.

Recently, scanning laser Doppler flowmetry (SLDF) has been reported to measure blood flow directly in the ONH, in a rapid and noninvasive fashion. The technique is based on the Doppler effect, in which moving red blood cells cause a shift in the frequency of the reflected laser beam.[18]

In 1998, Michelson and coworkers[19] described a new method for SLDF automatic full-field perfusion image analysis and reported intersession coefficients of reliability for the flow parameter in the nasal and temporal peripapillary retina of 0.76 and 0.73, respectively, in normal subjects.

The aim of the present study was to evaluate blood flow in the neuroretinal rim of the ONH and in the peripapillary retina using SLDF automatic full-field perfusion image analysis. Perfusion measurements using this technique are compared in open-angle glaucoma (OAG) patients, ocular hypertensives, and normal subjects, and then correlated with several clinical parameters.

METHODS

TWENTY UNCONTROLLED OAG PATIENTS (OAG GROUP) and 20 uncontrolled OHT patients (OHT group) were recruited from the glaucoma clinics of the hospital into this cross-sectional study. Twenty normal subjects (NOR group) were also recruited. An informed consent was obtained from all subjects.

Patients with OAG had gonioscopically open angles and fulfilled at least two of the following three criteria: history of IOP above 21 mm Hg, characteristic nerve fiber bundle visual field defects, and glaucomatous optic disk changes. Ocular hypertensives had a history of IOP above 24 mm Hg on at least two occasions, normal visual field, and an ONH appearance that ranged from normal to suspect but showed no localized thinning, saucerization, notching, or progression. Normal subjects had IOP below 21 mm Hg, normal visual field, and normal appearance of ONH with no characteristics suspicious of glaucomatous optic neuropathy. They also had normal ocular examinations and no family history of glaucoma. Open-angle glaucoma and OHT patients were considered uncontrolled when their current IOP was higher than their target IOP and were, therefore, candidates for further therapeutic intervention.

Measurements were performed on one eye of each subject. The study eye was chosen based on media clarity, larger neuroretinal rim area, and better fixation with the fellow eye. When there was no difference between eyes in these criteria, one eye was selected randomly. Subjects were excluded from the study if they had other abnormal ocular findings apart from pseudophakia, if significant media opacities or poor tear film quality precluded SLDF imaging, or if at any time they were unable to cooperate.

For each patient, medical and ocular history was taken. Intraocular pressures, best-corrected visual acuity, and refractive errors were measured. A routine ophthalmologic examination including biomicroscopy, gonioscopy, and ophthalmoscopy was performed, and a recent automated perimetry (Humphrey Field Analyzer, program 24-2 Humphrey Instruments, San Leandro, California, USA) was used to evaluate the visual field. Systemic blood pressure and heart rate were recorded, and ocular perfusion pressure was calculated according to the formula:

$$\text{OPP} = 2/3\,[\text{diastolic blood pressure} + 1/3\,(\text{systolic blood pressure} - \text{diastolic blood pressure})] - \text{IOP}$$

The scanning laser Doppler flowmeter used in this study (Heidelberg Retina Flowmeter [HRF], Heidelberg Engineering, GmbH, Heidelberg, Germany) is a noninvasive instrument combining both a laser Doppler flowmeter with a scanning laser technique. It measures the amount of backscattered light at different locations in the tissue of interest in a short period of time. Detailed descriptions of the instrument and measurement techniques have been previously published.[20,21] An infrared diode laser with wavelength of 780 nm and a power of 200 μW is used. The area examined measures 2.7 mm × 0.7 mm in size and is composed of 64 horizontal lines, each with 256 points giving an approximate spatial resolution of 10 μm. Each line is scanned sequentially 128 times with a total acquisition time of 2.05 seconds. A two-dimensional map of microvascular perfusion of the area to be studied is thus generated.

New scanning laser Doppler flowmetry analysis software (SLDF version 3.3) developed by Michelson and associates[19] enhances the computations generated by the HRF. The software excludes pixels with incorrect brightness (invalid pixels), marks saccades that lead to erroneous perfusion data, and eliminates pixels of retinal vessels with a diameter greater than 30 μm. The analysis is based on the average of all valid image points, and the perfusion map is divided into neuroretinal rim area, temporal peripapillary retinal area, and nasal peripapillary retinal area. Each area is analyzed separately. Pupils 3 mm in diameter or smaller were dilated using tropicamide 1% (Alcon, Fort

TABLE 1. Patient Characteristics (Mean ± SD, *P* Value)

	OAG Group (n = 20)	OHT Group (n = 20)	NOR Group (n = 20)	Significance $P < 0.05$
Age (yrs)	66.6 ± 10.8	57.2 ± 11.6	63.7 ± 9.8	.02*
Sex (M/F)	9/11	9/11	9/11	
IOP	22.2 ± 4.2	28.7 ± 3.9	16.9 ± 2.6	<.0001*
IOP Max	28.8 ± 6.2	30.3 ± 3.3	-	.57†
C/D ratio	0.75 ± 0.2	0.41 ± 0.2	0.23 ± 0.1	<.0001*
M.D.	−9.94 ± 8.3	−0.38 ± 2.4	-	.00†
OPP	43.2 ± 6.1	42.8 ± 10.6	48.2 ± 7.2	.08*

C/D ratio = cup/disk ratio; IOP = intraocular pressure (mm Hg); IOP Max = maximum recorded intraocular pressure (mm Hg); M.D. = mean defect of visual field; NOR = normal; OAG = open-angle glaucoma; OHT = ocular hypertension; OPP = calculated ocular perfusion pressure.

*One-way ANOVA and Sidak multiple comparisons.

†Nonparametric analysis: (Mann-Whitney *U* test).

Worth, Texas, USA). The fundus camera was adjusted until a focused, evenly illuminated, and centered view of the ONH was obtained. The patient was asked to use the fellow eye for fixation and to refrain from movement and blinking during image acquisition. Using a 2.5-degree × 10- degree frame, a total of seven to nine images were then acquired in one session, focusing on the superficial retina. The angulation of the fundus camera as well as its distance from the eye was kept constant throughout the imaging session. All images were reviewed by the same observer, and, before data analysis, the best five images in terms of focusing, centration, brightness, and absence of movements were chosen. Patients whose images were considered to be of poor quality or unsuitable for analysis were excluded from the study. Full-field perfusion analysis was then performed on each of the five chosen images, and mean values of blood flow for the neuroretinal rim of the ONH, the temporal peripapillary retina, and the nasal peripapillary retina were obtained in arbitrary units (au).

Mean flow measurements were first transformed (square root transformation) to normalize distributions. One-way analysis of variance (ANOVA) was then used to compare mean values of the three study groups (OAG, OHT, and NOR). Statistical significance was set at $P < .05$. Significant analyses were followed by Sidak multiple comparison tests to locate differences. Flow values of the neuroretinal rim of the ONH, nasal peripapillary retina, and temporal peripapillary retina were then correlated with the clinical parameters of age, error of refraction, cup/disk (C/D) ratio, IOP, visual field mean defect, maximum-recorded IOP, and ocular perfusion pressure using Pearson correlation. Statistical significance was set at $P < .01$.

The position of the surface of the neuroretinal rim relative to the dominant focal plane, the peripapillary retina, may be different in each of the three study groups, and this difference may affect perfusion results. We therefore used the tomography capability of the combined Heidelberg retinal flowmeter/tomograph to retrospectively evaluate the magnitude and significance of these positions. Mean topographies were calculated from three high-quality Heidelberg retina tomograph (Heidelberg Engineering, Heidelberg, Germany) images of the ONH and peripapillary retina, obtained at the time of the SLDF session based on techniques previously described[22–26] and using software version 2.01. When changes in the position of the reference plane were accounted for, the location of the rim is determined by its average Z coordinate (that is, its average location relative to the mean peripapillary retinal surface height) as described in our previous study.[27] A positive value of the mean rim Z coordinate means that, on average, the rim surface is located posterior to the peripapillary retinal surface, that is, defocused posteriorly, whereas a negative value of the mean rim Z coordinate means that, on average, the rim surface is located anterior to the peripapillary retinal surface, that is, defocused anteriorly.

RESULTS

SCANNING LASER DOPPLER FLOWMETRY IMAGING WAS performed on 32 patients with OAG, 24 patients with OHT, and 29 normal subjects. Good quality images were obtained from 22 OAG patients, 20 OHT patients, and 21 normal subjects and were analyzed. From these, two OAG patient and one normal subject were excluded for invalid rim data. Data are, thus, presented for 20 OAG patients, 20 OHT patients, and 20 normal subjects. Causes of poor-quality images were primarily excessive eye movements and media opacities. Invalid rim data was a term generated by the software in cases where the extremely low reflectivity of the ONH, sometimes accompanied by high C/D ratio, caused inadequate number of pixels in the neuroretinal rim area to be available for analysis. The two OAG patients excluded for invalid rim data had C/D ratio > 0.8, whereas the normal subject (with C/D ratio of 0.4) had an unusually low reflectivity of the neuroretinal rim. All three subjects were excluded by the software.

Table 1 summarizes the characteristics of the three study

TABLE 2. Scanning Laser Doppler Flowmetry Measurements of the Neuroretinal Rim of ONH, Temporal Peripapillary Retina, and Nasal Peripapillary Retina of the OAG, OHT, and NOR groups

Location	OAG Group n = 20	OHT Group n = 20	% Diff. OAG vs OHT	NOR Group n = 20	% Diff. OAG vs NOR	*P* Value
Neuroretinal rim of ONH	157 ± 78	276 ± 157	−43%	272 ± 92	−37%	.001
Temporal peripapillary retina	316 ± 83	309 ± 78	2%	307 ± 85	3%	.93
Nasal peripapillary retina	303 ± 104	287 ± 104	6%	279 ± 79	9%	.76

NOR = normal; OAG = open-angle glaucoma; OHT = ocular hypertension; ONH = optic nerve head; % Diff. = percentage difference. One-way analysis of variance at 0.05 level.

groups. Statistically significant differences were demonstrated between the groups in clinical C/D ratio, IOP, and visual field mean defect.

Six OAG patients (30%), four OHT patients (20%), and two normal subjects (10%) had systemic hypertension. Two OAG patients (10%), one OHT patient (5%), and one normal subject (5%) had diabetes mellitus. Patients were allowed to continue on their prescribed systemic medications as well as their topical antiglaucoma medications. At the time of imaging, six of the twenty patients (30%) in the OAG group were on systemic therapy (angiotensin-converting enzyme [ACE], inhibitors 4 [20%]; β-blockers, 1 [5%]; and diuretics, 1 [5%]). Five of the 20 patients (25%) in the OHT group were on systemic therapy (ACE inhibitors, 2 [10%]; anticoagulants, 1 [5%]; and diuretics, 2 [10%]). Two of the 20 patients (10%) in the NOR group were on systemic therapy (β-blockers). Seventeen of the 20 patients (85%) in the OAG group and 11 of the 20 patients (55%) in the OHT group were on topical antiglaucoma medications whether monotherapy or combinations (OAG: β-blockers, 15 [75%]; α-adrenergics, 5 [25%]; cholinergics, 4 [20%]; CAIs, 10 [50%]) (OHT: β-blockers, 10 [50%]; α-adrenergics, 2 [10%]; cholinergics, 1 [5%]). Three patients (15%) in the OAG group had previous laser trabeculoplasty in the study eye. One OAG patient (5%) had a remote combined cataract and glaucoma surgery, and five normal subjects (25%) had a remote cataract surgery in the study eye.

Table 2 and Figure 1 summarize the SLDF blood flow measurements (mean ± standard deviation [SD]) for each study group; OAG patients demonstrated significantly lower blood flow values in the neuroretinal rim compared with OHT patients (−43%) and with normal subjects (−37%) (P = .001, one-way ANOVA). No statistically significant difference in flow values was observed in the neuroretinal rim between the OHT group and the NOR group (P = .91, Student t test). Furthermore, no statistically significant difference in flow values was observed between the three groups in temporal and nasal peripapillary retina (P = .93 and .76 respectively, one-way ANOVA).

Three age-matched subgroups of 15 OAG, 15 OHT, and 15 NOR subjects were then compared in order to exclude the influence of age on our results. In these subgroups, OAG patients had a mean (± SD) age of 63 ± 10 years, OHT patients had a mean age of 61 ± 11 years, and NOR subjects had a mean age of 63 ± 11 years. In the OAG subgroup, mean rim flow was 143 ± 85 au. Conversely, the OHT subgroup had a mean rim flow of 313 ± 161 au (P = .0017, Student t test), whereas the NOR subgroup had a mean rim flow of 280 ± 101 au (P = .0004, Student t test). Peripapillary retinal flow showed no significant difference between groups ($P \geq .577$).

We also matched eight OAG patients and eight OHT subjects for IOP. Both groups had a mean (± SD) IOP of 26 ± 3 mm Hg. In the OAG subgroup mean rim flow was 126 ± 73 au, whereas in the OHT subgroup mean rim flow was 300 ± 222 au (P = .066, Student t test). Temporal and nasal peripapillary retinal blood flow showed no significant difference between the two subgroups (P = .505 and 0.251, respectively). Similarly, eight OAG patients and eight NOR subjects were matched for IOP. Both groups had IOP of 18 ± 2 mm Hg. In the OAG subgroup mean rim flow was 188 ± 88 au, whereas in the NOR subgroup mean rim flow was 341 ± 90 au (P = .004, Student t test). Temporal and nasal peripapillary retinal blood flow showed no significant difference between the subgroups (P = .058 and .825, respectively).

The decreased mean rim flow in the OAG subgroups compared with each of the OHT and NOR subgroups show that neither age nor IOP have influenced the observed perfusion difference between the three study groups.

A correlation matrix was then established for our study population (n = 60) between blood flow values of the neuroretinal rim, nasal, and temporal peripapillary retina vs the clinical parameters age, C/D ratio, IOP, visual field mean defect, maximum-recorded IOP, and ocular perfusion pressure (Table 3). Cup/disk ratio was the only parameter that showed a significant inverse correlation with neuroretinal rim blood flow ($r = -0.415$, P = .001, Pearson's correlation). Neuroretinal rim blood flow showed no such correlation with the other parameters examined ($P \geq .13$). Peripapillary retinal blood flow showed no significant correlation with any clinical parameter ($P \geq .14$).

Correlation between neuroretinal rim flow and these parameters was then calculated for each of the three study groups to test if such a correlation was present within each

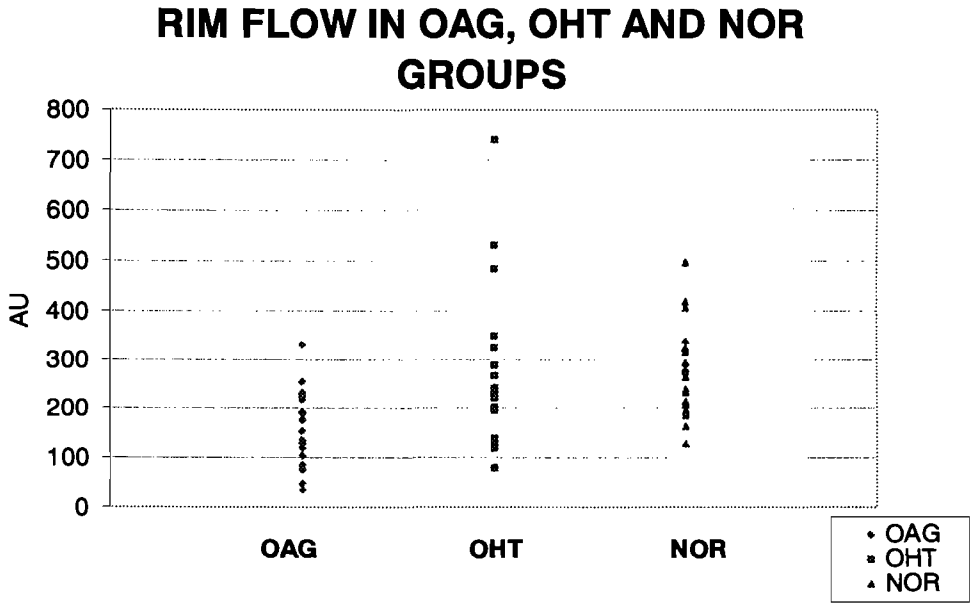

FIGURE 1. Scattergrams of scanning laser Doppler flowmetry blood flow measurements for the neuroretinal rim of the optic nerve head in the open-angle glaucoma (OAG) group, ocular hypertension (OHT) group, and normal (NOR) group. One-way analysis of variance at 0.05 level. AU = arbitrary units.

TABLE 3. Correlation Between Blood Flow in the Neuroretinal Rim of ONH, Nasal Peripapillary Retina, and Temporal Peripapillary Retina vs Various Clinical Parameters (n = 60)

	Neuroretinal Rim of ONH		Nasal Peripapillary Retina		Temporal Peripapillary Retina	
Parameters	R Value	*P* Value	R Value	*P* Value	R Value	*P* Value
Age	.138	.29	.069	.60	−.073	.58
IOP	.001	.99	−.075	.57	−.013	.92
C/D ratio	−.415	.00	.192	.14	.124	.35
M.D.*	.247	.13	−.193	.24	−.091	.58
10P Max*	.153	.36	−.080	.63	.111	.51
OPP	−.010	.94	.131	.32	−.060	.65

C/D = cup/disk ratio; IOP = intraocular pressure (mm Hg); M.D. = mean defect of visual field; ONH = optic nerve head; OPP = calculated ocular perfusion pressure; 10P Max = maximum recorded intraocular pressure (mm Hg).
Pearson correlation—significance (two-tailed) at 0.01 level.
*n = 40.

group, but the sample size was too small to show any significant correlation. However, there was an inverse but statistically nonsignificant relationship between neuroretinal rim blood flow and C/D ratio in the OHT group ($r = -0.363$, $P = .115$, Pearson correlation).

To better understand the relationship between C/D ratio and neuroretinal rim blood flow in the OHT group, we split the OHT group into two equal subgroups: 10 subjects having a C/D ratio equal to or greater than 0.4 (mean, 0.54 ± 0.13) and 10 subjects having a C/D ratio less than 0.4 (mean, 0.27 ± 0.07); OHT eyes with C/D ratio ≥ 0.4 (n = 10) showed significantly lower a mean neuroretinal rim flow compared with OHT eyes with C/D ratio < 0.4 (n = 10) (203 ± 79 au vs 350 ± 185 a C/D au, $P = .039$, Student *t* test) (Figure 2).

The OHT eyes with C/D ratio ≥ 0.4 showed a significantly lower mean neuroretinal rim flow compared with the NOR group (203 ± 79 au vs 272 ± 93 au, $P = .047$), whereas no statistically significant difference was shown compared with the OAG group (203 ± 79 au vs 158 ± 79 au, $P = .156$). Conversely, OHT eyes with C/D ratio < 0.4 showed significantly higher mean a neuroretinal rim flow compared with the OAG group (350 ± 185 au vs 158 ± 79 au, $P = .010$), whereas no statistically significant difference was shown compared with the NOR group (350 ± 185 au vs 272 ± 93 au, $P = .232$).

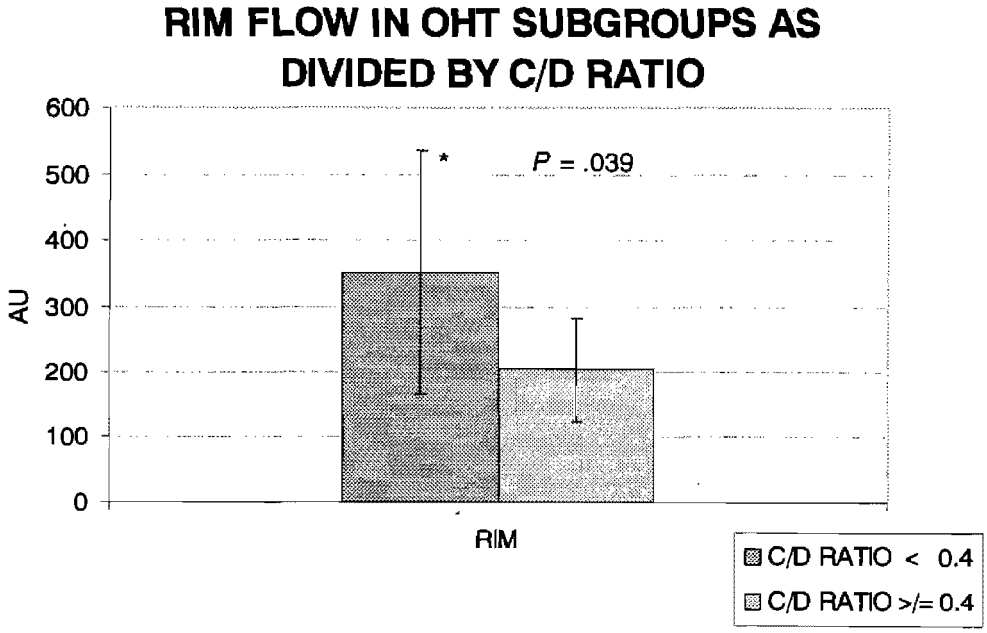

FIGURE 2. Scanning laser Doppler flowmetry blood flow measurements (mean ± standard deviation) for the neuroretinal rim of the optic nerve head (ONH) in the ocular hypertension (OHT) group as divided into two equal subgroups based on cup-to-disk ratio (C/D ratio). Student *t* test at 0.05 level. RIM = neuroretinal rim of the ONH. AU = arbitrary units.

Proper interpretation of SLDF perfusion images requires optimized technical settings specifically as regards reflectivity and focusing. Differences in reflectivity and focusing among study groups may introduce bias in analysis, especially if thinner rim/larger cup is related to incorrect brightness and/or differences in focusing.

The SLDF full-field perfusion analysis software[19] assumes adequate brightness. To meet this requirement, pixels with incorrect brightness, that is, underexposed and overexposed pixels, are excluded from analysis. Differences in percentage of eliminated pixels among groups may introduce bias in the analysis. Therefore, it is important to know the percentage of pixels used by the software in each study group and whether there were differences among groups with respect to these percentages.

Compared with the total number of pixels in the scan area, the percentage of neuroretinal rim area pixels was significantly different between the three study groups (12.7% ± 6.3% in the OAG group, 23.5% ± 7.4% in the OHT group, and 30.4% ± 6.2% in the NOR group) ($P < .0001$, one-way ANOVA). This is a clear consequence of the OAG group having a smaller rim compared with the other groups. However, the percentage of valid pixels in the rim area (that is, pixels used by the software for perfusion analysis) showed little difference among the three study groups (15.1% ± 17.0% in the OAG group, 18.0% ± 19.4% in the OHT group, and 18.7% ± 11.1% in the NOR group). This difference was not statistically significant ($P = .681$, one-way ANOVA).

To determine whether there was a relationship between the number of valid rim pixels and the rim flow values in glaucoma patients, we performed a comparison between neuroretinal rim blood flow in glaucoma patients with larger (upper third, n = 7) number of valid pixels vs smaller (lower third, n = 7) number of valid pixels. We found no significant difference in neuroretinal rim blood flow between these two subgroups ($P = .85$, Student *t* test). Decreased neuroretinal rim blood flow in glaucoma patients was not related to the number or percentage of valid pixels used for perfusion analysis.

Differences in mean rim height relative to the peripapillary retina were evaluated in each of the three study groups. Both the OAG and the OHT groups demonstrated a mean posterior displacement in the neuroretinal rim surface of 104 ± 94 μm and 64 ± 119 μm, respectively, whereas the NOR group demonstrated an anterior displacement of 23 ± 89 μm ($P = .021$, one-way ANOVA).

DISCUSSION

THIS STUDY DEMONSTRATES THAT, AS MEASURED WITH scanning laser Doppler flowmetry and full-field perfusion analysis, OAG patients have significantly lower blood flow in the neuroretinal rim of the ONH when compared with OHT patients and normal subjects. Peripapillary retinal blood flow did not show such a difference between the three study groups. The reduction in neuroretinal rim

blood flow was significantly correlated with the increasing C/D ratio.

Our results confirm the findings of several studies that reported a significantly reduced retro-ocular and bulk choroidal blood flow and increased vascular resistance in OAG compared with normal subjects and ocular hypertensives.[28-42] Using fluorescein angiography, Wolf and associates[43] demonstrated that eyes with OAG are associated with an increased arteriovenous passage time and a decreased dye velocity. Using single-point laser Doppler flowmetry, several authors similarly reported decreased blood flow in the ONH of OAG when compared with control subjects[44] and glaucoma suspects.[45]

Scanning laser Doppler flowmetry has been used in several studies comparing flow measurements of OAG patients and normal subjects. Michelson and associates[46] reported that both neuroretinal rim blood flow and peripapillary retinal blood flow were significantly decreased in OAG patients compared with age-matched controls. Nicolela and associates[47] reported a significant decrease in blood flow in the lamina cribrosa and the upper temporal, but not the lower temporal, peripapillary retina in OAG patients compared with control subjects. No difference in flow measurements of the neuroretinal rim was found. Findl and associates,[48] using both SLDF and fundus pulsation amplitudes, similarly reported reduced blood flow in the disk cup (−46%) and the neuroretinal rim (−18%) in patients with OAG compared with age-matched control subjects. Conversely, Hollo and associates[49] failed to detect significant differences in neuroretinal rim blood flow or in peripapillary retinal blood flow[50] in their population of OAG patients and NTG patients compared with control subjects.

Scanning laser Doppler flowmetry has also been used to compare ONH and retinal perfusion of OAG patients and ocular hypertensives. Kerr and associates[51] reported reduced blood flow in the lamina cribrosa and the temporal neuroretinal rim of the ONH of glaucoma patients in comparison with ocular hypertensives. However, no difference was found between groups at the nasal neuroretinal rim or nasal peripapillary retina, and an increase in minimum velocity was reported at the temporal peripapillary retina in the glaucoma group.

Our results confirm the findings of Michelson and associates[46] as regards reduced blood flow in the neuroretinal rim of glaucoma patients compared with controls and those of Kerr and associates[51] as regards reduced blood flow in the neuroretinal rim of glaucoma patients compared with ocular hypertensives. However, we could not demonstrate significant differences in ONH blood flow between the OHT group and NOR group. For peripapillary retinal blood flow, no significant difference was seen between the three study groups. We believe that the reason for such discrepancies might be that all the previous studies used for analysis the conventional evaluation of 10 × 10 pixel measurement box that was reported to show several technical limitations including:[52]

1. Variation in location of the measurement box selected by the operator between images. The slightest difference in the location of the measurement box in relation to retinal or ONH vessels may lead to considerable variation of perfusion values between images.
2. Placement of a large measurement box on the neuroretinal rim of glaucoma patients with high C/D ratio and thin rim unavoidably includes an area of the cup or the peripapillary retina in the box, thus leading to erroneous data.
3. The appropriate illumination of the ocular tissue during image acquisition taking into consideration the low reflectivity of the ONH compared with the peripapillary retina and the influence of brightness on the measurements of the SLDF.
4. Differences in focusing between the neuroretinal rim and peripapillary retina vs the lamina cribrosa. Different focal planes should be used for evaluation of cup and rim perfusion.

Using the 10 × 10 pixel measurement box, reproducibility of the neuroretinal rim flow was considered poor, with an intersession coefficient of reliability of 0.36 in glaucoma patients and 0.47 in normal volunteers[53] although peripapillary retinal flow appeared to be more reproducible.[27,53,54]

In the present study we utilized the SLDF full-field perfusion analysis software (version 3.3). The software was reported to provide higher reproducibility values than those reported using the 10 × 10 pixel measurement box.[25] Iester and associates,[55] using the same software, reported good intraobserver reproducibility (intra-image and inter-image). However, the reproducibility was still significantly better in the peripapillary retina than in the rim area. We have demonstrated that obtaining mean values from five high-quality images further improves the reproducibility of the technique and were able to report an intrasession coefficient of reliability that ranged from 0.93 to 0.99 and an intersession coefficient of reliability that ranged from 0.80 to 0.99 in our population of glaucoma patients and normal volunteers.[56]

This study is also unique in its examination of all three pertinent groups, OAG, OHT and normals. Our study groups included uncontrolled OAG and OHT patients, some of whom were newly diagnosed and receiving no treatment and others who were uncontrolled despite topical antiglaucoma medications. The heterogeneity of the medications and the combined therapies gave very small groups, which precluded statistical evaluations; however, no specific trend was apparent. Comparisons of blood flow values between patients using antiglaucoma medications and patients not on therapy could not be performed because the sample size was too small.

We observed an inverse correlation between neuroretinal rim flow values and C/D ratio (−0.415, $P = .001$),

which is suggestive of a link between defective perfusion of the ONH and severity of glaucomatous optic disk changes. The correlation with C/D ratio was across all three groups, and, in contrast, there was no similar correlation between neuroretinal rim flow values and visual field mean defect, suggesting that rim blood flow was correlated with C/D ratio rather than with patient's diagnosis (or perimetric status). A similar correlation between ONH blood flow and C/D ratio was reported by Michelson and associates[46] in glaucoma patients.

The OHT group had a range of C/D ratios from 0.15 to 0.70 (mean, 0.41 ± 0.2). Mean neuroretinal rim blood flow was lower in OHT patients with larger C/D ratios compared with OHT patients with smaller C/D ratios (P = .039, Student t test) (Figure 2). A similar correlation was reported by Piltz-Seymour and associates[45] in their evaluation of ONH perfusion in glaucoma suspects using single-point laser Doppler flowmetry.

Changes in focus might artifactually change SLDF measurements. Perfused tissue located significantly anterior or posterior to the focal plane has been shown to yield artifactually higher SLDF perfusion values. In recent studies, which we have confirmed in our laboratory (data not shown)[57,58], it was shown that as the focal plane is moved either anterior or posterior to the surface of rim tissue, measured values artifactually increase. Prokopich and associates reported that a focal plane displacement of 200 μm (0.5 diopters) was found to result in an artifactual change in perfusion of 30 au.[59]

We have therefore meticulously established the optimal focal plane during our imaging sessions using the tomography capability of the combined flowmeter/tomograph. We have also retrospectively evaluated the position of the surface of the neuroretinal rim with respect to the dominant focal plane, the surface of the peripapillary retina, in each of the three study groups. Because most OAG patients and some OHT patients show anteroposterior thinning of the neuroretinal rim, one would expect that the rim surface would commonly be located posterior to the surface of the peripapillary retina. Our analysis demonstrates a posterior defocusing of the rim relative to the mean peripapillary retinal surface of 104 μm in the OAG group and 64 μm in the OHT group, whereas the NOR group demonstrates an anterior defocusing of 23 μm. This posterior defocusing of the neuroretinal rim in the OAG group would have manifested as an increase (rather than a decrease) in rim perfusion values in the range of 15 au, which is considered too small to have significantly altered our findings.

In conclusion, we have demonstrated defective perfusion in the neuroretinal rim of the ONH in glaucoma patients compared with ocular hypertensives and normal subjects, as measured by SLDF full-field perfusion analysis. We were not able to attribute such defective perfusion to the use of topical or systemic medications. It was also not established whether these changes precede or result from glaucomatous optic disk changes. A definite inverse correlation between reduced neuroretinal rim flow values and higher C/D ratio was also established. This correlation was apparent, though not significant, within the OHT group, suggesting that neuroretinal rim perfusion may be reduced in high-risk ocular hypertensives before the manifestation of visual field defects.

We believe our data may lay the groundwork for a long-term study to examine whether OHT patients with lower rim perfusion values are more likely to show progression to glaucoma, whether we can distinguish between OHT patients needing treatment vs those that do not based on rim perfusion values, and, finally, whether ONH perfusion might be a prognostic marker for future stability in both OAG and OHT patients. This will eventually lead to better understanding of the disease and better therapy for our patients.

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Changes in Optic Nerve Head Blood Flow after Therapeutic Intraocular Pressure Reduction in Glaucoma Patients and Ocular Hypertensives

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Purpose: To detect and quantify changes in optic nerve head (ONH) and peripapillary retinal blood flow by scanning laser Doppler flowmetry (SLDF) in open-angle glaucoma (OAG) and ocular hypertension (OHT) after therapeutic intraocular pressure (IOP) reduction.

Design: Prospective, nonrandomized, self-controlled trial.

Participants: Twenty patients with OAG and 20 patients with OHT with clinical indications for therapeutic IOP reduction were prospectively enrolled.

Intervention: IOP reduction was achieved by medical, laser, or surgical therapy. All patients had IOP reductions more than 20% and a minimum of 4 weeks follow-up.

Main Outcome Measures: Blood flow measurements were performed by SLDF analysis software (version 3.3) using Heidelberg Retina Flowmeter images. Statistical evaluations were performed on both groups using a two-tailed distribution paired *t* test.

Results: Twenty patients with OAG had a mean IOP reduction of 37% after treatment. In these patients, mean (± standard deviation) rim blood flow increased by 67% (from 158 ± 79 arbitrary units to 264 ± 127 arbitrary units, $P = 0.001$), whereas mean temporal peripapillary retinal flow decreased by 7.4% ($P = 0.24$), and mean nasal peripapillary retinal flow increased by 0.3% ($P = 0.96$). Twenty OHT patients had a mean IOP reduction of 33% after treatment. In contrast to the OAG group, neither the mean rim blood flow (7.5% increase from 277 ± 158 arbitrary units to 298 ± 140 arbitrary units, $P = 0.41$) nor the mean temporal ($P = 0.35$) or nasal ($P = 0.88$) peripapillary retinal flow changed significantly.

Conclusions: For a similar percentage of IOP reduction, OAG patients had a statistically significant improvement of blood flow in the neuroretinal rim of the ONH, whereas OHT patients did not demonstrate such a change. Peripapillary retinal blood flow, expected to be affected less in glaucoma, remained stable in both groups. In addition to indicating a response to therapy in OAG patients, the reported changes in rim perfusion suggest that ONH autoregulation may be defective in OAG while intact in OHT. *Ophthalmology 2003;110: 201–210 © 2003 by the American Academy of Ophthalmology.*

The pathogenesis of optic nerve damage in glaucoma is still not fully understood. There is substantial evidence indicating that glaucomatous optic neuropathy is multifactorial in nature, with elevated intraocular pressure (IOP) being the most common risk factor. However, vascular factors have been postulated to play a major role.[1–3] These factors include autoregulation of blood flow in the optic nerve head (ONH) and other ocular tissues, local vasospasm, arterial hypertension, and nocturnal hypotension.

Although such vascular factors have been studied for decades, only recent technical developments have enabled noninvasive investigations of the associated circulatory disturbances. These investigations point to defective ONH blood flow as a likely contributing factor in the development of glaucomatous optic neuropathy.[2,3] Consequently, it could be assumed that ONH blood flow might improve after

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institution of therapy that would control the IOP and stabilize the glaucomatous optic neuropathy.

ONH blood flow depends on ocular perfusion pressure, which can be defined as the mean arterial blood pressure in the ocular vessels minus the intraocular pressure. Thus, in the absence of autoregulation, there is an inverse relationship between IOP and ocular perfusion pressure. The higher the IOP, the lower the ocular perfusion pressure, and consequently the lower the blood flow in the ONH. Conversely, reduction of IOP would be expected to improve ocular perfusion pressure and consequently increase ONH blood flow.[4]

The purpose of autoregulation in the ONH is to maintain a relatively constant blood flow despite changes in ocular perfusion pressure. The existence of intact autoregulation in the normal ONH has been demonstrated in a large number of experimental[5–9] and clinical[10–13] studies.

Autoregulation is reported to operate only within a critical range of ocular perfusion pressures and becomes ineffective when the ocular perfusion pressure goes below or above this critical range.[4] This range of ocular perfusion pressures has been investigated in different species using various methods.[5–7,14–17] Geijer and Bill[5] reported autoregulation of the ONH to be normal at an ocular perfusion pressure of >30 mmHg. Ernest[14] reported similar findings with pressures >50 mmHg. Breakdown of autoregulation was reported to take place at <30 mmHg by Bill and Sperber,[15] at <25 mmHg by Sossi and Andersen,[6] and at 30 to 35 mmHg perfusion pressure by Hayreh and coworkers.[17]

It is also hypothesized that glaucomatous optic neuropathy may be due to an eventual breakdown in ONH autoregulation.[10,11,18,19] If this hypothesis was correct, then similar changes in IOP, while accompanied by changes in ONH blood flow in glaucoma patients, would not be associated with such changes in subjects lacking glaucomatous optic neuropathy. We set out to test this hypothesis on patients with open-angle glaucoma (OAG) and ocular hypertension (OHT). Our study was performed in a true clinical context on patients who required therapeutic IOP reductions.

We performed our measurements using scanning laser Doppler flowmetry (SLDF). This system permits direct quantitative measurements of ONH and peripapillary retinal perfusion.[20–22] It has been reported to give both valid[26,27] and reproducible[20,21,23–25] results.

The aim of this study is to detect and quantify changes in ONH and peripapillary retinal blood flow using SLDF full-field perfusion analysis in patients with OAG and OHT undergoing therapeutic IOP reduction by medical, laser, or surgical intervention.

Patients and Methods

The study was approved by the research committee of Maisonneuve-Rosemont Hospital, University of Montreal. Informed consent was obtained from each patient before enrollment in the study.

Twenty patients with OAG and 20 patients with OHT were recruited from the glaucoma clinics of the hospital into this prospective study. Only patients achieving a minimum of 20% IOP reduction were eligible to complete the study.

Patients with OAG had glaucomatous optic neuropathy, characteristic nerve fiber bundle visual field defects, and gonioscopically open angles with no restrictions for IOP. OHT subjects had a history of repeated IOPs greater than 24 mmHg with normal visual fields and normal or suspect ONH appearance. Subjects were excluded from the study if they had abnormal ocular findings other than pseudophakia, if they had significant media opacities precluding SLDF imaging, or if they were unable to cooperate.

During the prestudy visit of each patient, medical and ocular history was recorded. IOPs, best-corrected visual acuity, and refractive errors were measured. A routine ophthalmologic examination including biomicroscopy, gonioscopy, and ophthalmoscopy was performed. A recent automated perimetry (Humphrey Field Analyzer, Program 24-2, Humphrey Instruments, San Leandro, California) was used to evaluate the visual field. Systemic arterial blood pressure and heart rate were recorded. Ocular perfusion pressure was calculated according to the formula[4]: Ocular perfusion pressure = 2/3 (Diastolic blood pressure + 1/3 [systolic blood pressure − diastolic blood pressure]) − IOP. Statistical analysis of patients' characteristics and perfusion parameters was performed using the two-tailed Student's t test ($P < 0.05$).

The SLDF used in this study (Heidelberg Retina Flowmeter [HRF], Heidelberg Engineering, GmbH, Heidelberg, Germany) is a noninvasive instrument combining both a laser Doppler flowmeter with a scanning laser technique. It measures the amount of backscattered light at different locations in the tissue of interest in a short period of time. An infrared diode laser with a wavelength of 780 nm is used. The area examined measures 2.7 mm × 0.7 mm and is composed of 64 horizontal lines, each with 256 points, giving an approximate spatial resolution of 10 μm. Each line is scanned sequentially a total of 128 times with a total acquisition time of 2.05 seconds. A two-dimensional map of microvascular perfusion of the area to be studied is thus generated.

New SLDF analysis software (version 3.3) developed by Michelson and associates[22] enhances the computations generated by the HRF. The software excludes pixels with incorrect brightness, marks saccades that lead to erroneous perfusion data, and eliminates pixels of retinal vessels with a diameter greater than 30 μm. The analysis is based on the average of all valid image points with the perfusion map divided into neuroretinal rim area, temporal peripapillary retinal area, and nasal peripapillary retinal area. Each area is analyzed separately (Fig 1).

We have recently demonstrated that SLDF full-field perfusion analysis produces highly reproducible intrasession and intersession measurements of ONH and peripapillary retinal blood flow in our population of glaucoma patients and normal volunteers (Bizzarro et al [Invest Ophthalmol Vis Sci 2000;41(4):S556]). Using a mean of five images, our intersession reproducibility in glaucoma patients was 0.87 to 0.99, depending on the location of measurement.

Image Acquisition Technique

Pupils 3 mm in diameter or smaller were dilated. The fundus camera was adjusted until a focused, evenly illuminated and centered view of the ONH was obtained. The patient was asked to use the fellow eye for fixation and to refrain from movement and blinking during image acquisition. Using a 2.5° × 10° frame, 7 to 10 images were then acquired in one session, focusing on the superficial retina.

All images were reviewed by the same observer, and before data analysis, the best five images in terms of focusing, centration, brightness, and absence of movements were chosen. Patients whose images were considered to be of poor quality or unsuitable for analysis were excluded from the study. SLDF full-field perfusion analysis was then performed on each of the five images with measurements for flow, volume, and velocity given in arbitrary

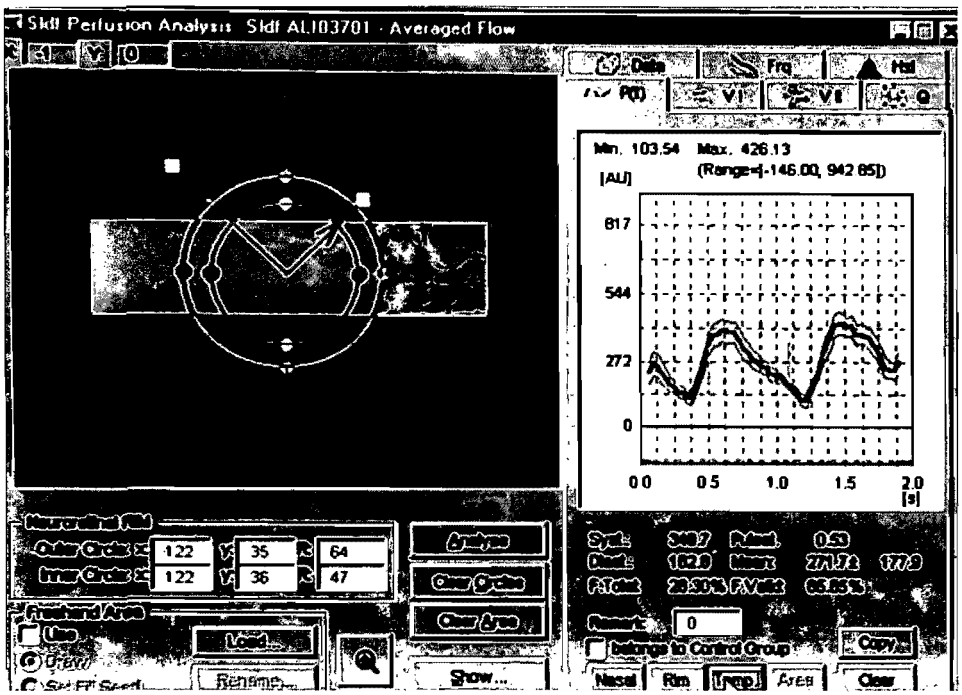

Figure 1. Flow image of temporal peripapillary retinal area using the scanning laser Doppler flowmetry full-field perfusion analysis showing the outline of the neuroretinal rim area (left) and a graphic presentation of the heartbeat-associated pulsation of capillary blood flow (right).

units for the neuroretinal rim area, temporal peripapillary retinal area, and nasal peripapillary retinal area.

All patients then underwent IOP reduction by medical, laser, or surgical intervention. All patients included in the study had a sustained IOP reduction of 20% or more, as well as a minimum of 4 weeks follow-up between the intervention and the second SLDF session.

Patients then underwent a second session of SLDF imaging using the same settings previously used for image acquisition (scan area, focusing, sensitivity) and full-field perfusion analysis (position of the rim circles, calculation parameters). The best five images were similarly chosen and analyzed. A mean of the five readings for the parameters flow, volume, and velocity was then computed for each of the two sessions.

Statistical evaluations were performed on both OAG and OHT groups using two-tailed paired distribution *t* test for the parameters flow, volume, and velocity. Statistical significance was set at $P < 0.05$.

Perfused tissue located significantly anterior or posterior to the focal plane has been shown to yield artifactually higher SLDF values (Lundmark et al [Invest Ophthalmol Vis Sci 1996;37:S265] and Segawa et al [Invest Ophthalmol Vis Sci 1997;38:S774]). Because it has also been shown that ONH morphology changes after reduction of IOP,[28–30] we assessed the changes in the position of the surface of the neuroretinal rim relative to the dominant focal plane, the peripapillary retina, using confocal scanning laser ophthalmoscopy both before and after IOP reduction. Mean topographies were calculated from three high-quality Heidelberg Retina Tomograph (HRT, Heidelberg Engineering, Heidelberg, Germany) images of the ONH and peripapillary retina, obtained at the time of each of the two SLDF sessions, based on techniques previously described[31–35] and using software version 2.01.

Mean rim height, the average height of the rim surface above the reference plane, was calculated using the equation:

$$\text{Mean rim height} = \text{Volume above reference plane/rim area}$$

where values for volume above reference plane and rim area are given by the HRT software in the tilted relative coordinate system.

When changes in the position of the reference plane were accounted for, the mean rim height would be its average Z coordinate (i.e., its average location relative to the mean peripapillary retinal surface height). This value can be calculated using the equation:

$$\text{Mean rim Z coordinate} = \text{Reference height} - \text{Mean rim height}$$

where values for the reference height are given by the HRT software, and the mean rim height is calculated as described previously.

The mean rim Z coordinate can be positive or negative. A positive value means that, on average, the rim surface is located posterior to the peripapillary retinal surface, whereas a negative value means that, on average, the rim surface is located anterior to the peripapillary retinal surface

Results

Pre-IOP and post-IOP reduction SLDF imaging was performed on 32 patients with OAG and 24 patients with OHT. Good-quality images were obtained from 22 OAG and 20 OHT patients and were analyzed. From these, two OAG patients were excluded for invalid rim data. Data are thus presented for 20 OAG and 20 OHT patients.

Causes of poor quality images were primarily excessive eye movements and media opacities. Invalid rim data was the term generated by the software in two cases in which the extremely low reflectivity of the ONH, accompanied by high cup-to-disc ratio, caused an inadequate number of pixels in the rim area to be available for SLDF analysis.

The mean age (±standard deviation [SD]) was 66.7 ± 10.9 years for the OAG group (age range, 42–79 years) and 57.2 ± 11.6 years for the OHT group (age range, 42–75 years). There were 9 males (45%) and 11 females (55%) in each of the two study groups.

Table 1. Characteristics of Open-angle Glaucoma and Ocular

	Age (yrs)	Gender (M/F)	Intraocular Pressure before Reduction (mm Hg)	Maximum Intraocular Pressure Recorded (mm Hg)	Cup/ Disc Ratio	Mean Defect	Error of Refraction
OAG eyes (n = 20)	66.7 ± 10.9	9/11	22.2 ± 4.2	28.8 ± 6.2	0.75 ± 0.2	−9.94 ± 8.3	−0.9 ± 2.7
OHT eyes (n = 20)	57.2 ± 12.3	9/11	28.7 ± 3.9	29.8 ± 3.5	0.41 ± 0.2	−0.38 ± 2.4	+0.2 ± 2.8
t Test	0.011	—	0.000	0.359	0.000	0.000	0.186

OAG = open angle glaucoma; OHT = ocular hypertension.

Six OAG patients (30%) and four OHT patients (20%) had systemic hypertension. Two OAG patients (10%) and one OHT patient (5%) had diabetes mellitus. At the first imaging session, 6 of the 20 patients in the OAG group were receiving systemic therapy (angiotensin-converting enzyme inhibitors, 4 [20%]; β-blockers, 1 [5%]; diuretics, 1 [5%]). Five of the 20 patients (25%) in the OHT group were receiving systemic therapy (angiotensin-converting enzyme inhibitors, 2 [10%]; anticoagulants, 1 [5%]; diuretics, 2 [10%]).

Seventeen of the 20 patients in the OAG group were receiving topical glaucoma therapy, whether monotherapy or combinations (β-blockers, 15 [75%]; α-adrenergics, 5 [25%]; cholinergics, 4 [20%]; carbonic anhydrase inhibitors, 10 [50%]). Eleven of the 20 patients (55%) in the OHT group were receiving topical glaucoma therapy (β-blockers, 10 [50%]; α-adrenergics, 2 [10%]; cholinergics, 1 [5%]). Three patients (15%) in the OAG group had a previous laser trabeculoplasty. One OAG patient (5%) had a remote combined cataract and glaucoma surgery.

Table 1 summarizes the characteristics of the OAG and OHT study groups. There was no statistically significant difference in the means of maximum-recorded IOP, refractive error, and calculated ocular perfusion pressure between the two groups. Statistically significant differences were demonstrated between the OAG group and the OHT group in the means of age, IOP before reduction, clinical cup-to-disc ratio, and visual field mean defect ($P \leq 0.01$).

The mean percentage of IOP reduction was 36.9% in the OAG group and 32.7% in the OHT group (Table 1). There were no statistically significant differences between the two groups in the percentage of IOP reduction and the follow-up duration (P = 0.349 and 0.827, respectively). A statistically significant difference was demonstrated between the OAG group and the OHT group in the mean IOP after reduction (P = 0.0002).

Therapeutic IOP reduction was attained in 4 of the 20 OAG patients by ocular hypotensive drugs, whether monotherapy or combinations (β-blockers, 2 [10%]; α-adrenergics, 1 [5%]; cholinergics, 1 [5%], and carbonic anhydrase inhibitors, 2 [10%]). Twelve of the 20 OHT patients had therapeutic IOP reduction by ocular hypotensive drugs (β-blockers, 10 [50%]; α-adrenergics, 2 [10%], and carbonic anhydrase inhibitors, 3 [15%]).

Table 2 summarizes the SLDF perfusion data (mean ± SD, % change, P value) of the neuroretinal rim, temporal peripapillary retina, and nasal peripapillary retina of both the OHT and OAG groups before and after IOP reduction. Before IOP reduction, OAG patients demonstrated significantly lower blood flow, volume, and velocity values in the neuroretinal rim area compared with OHT patients ($P \leq 0.005$). No statistically significant difference in perfusion values was observed between OAG and OHT patients in the temporal and nasal peripapillary retina ($P \geq 0.79$ and $P \geq 0.63$, respectively).

After sustained IOP reduction, in the OAG group (Fig 2 and Table 2) mean rim blood flow showed a statistically significant

Table 2. Scanning Laser Doppler Flowmetry Perfusion Measurements (Mean ± Standard Deviation)

	Ocular Hypertension Group (n = 20)				Open-angle Glaucoma Group (n = 20)			
Location Parameter	*Perfusion Values Before Intraocular Pressure Reduction*	*Perfusion Values After Sustained Intraocular Pressure Reduction*	*% Change in Perfusion*	*P Value*	*Perfusion Values before Intraocular Pressure Reduction*	*Perfusion Values After Sustained Intraocular Pressure Reduction*	*% Change in Perfusion*	*P Value*
Neuroretinal rim								
FLO	276.8 ± 157.8	297.6 ± 139.7	**+7.5**	0.413	157.8 ± 78.9	263.9 ± 126.7	**+67.2**	0.001
VOL	20.3 ± 6.5	19.3 ± 4.9	**−4.9**	0.447	13.6 ± 4.2	15.4 ± 5.0	**+13.2**	0.091
VEL	1.33 ± 0.58	1.34 ± 0.51	**+0.8**	0.897	0.85 ± 0.35	1.12 ± 0.48	**+31.8**	0.006
Temporal PP retina								
FLO	309.0 ± 78.0	293.0 ± 68.0	**−5.2**	0.347	316.9 ± 83.4	293.3 ± 70.9	**−7.4**	0.240
VOL	20.5 ± 4.8	19.0 ± 4.6	**−7.3**	0.235	20.9 ± 5.9	18.3 ± 3.7	**−12.4**	0.063
VEL	1.10 ± 0.27	1.04 ± 0.23	**−5.5**	0.325	1.14 ± 0.31	1.05 ± 0.25	**−7.9**	0.180
Nasal PP Retina								
FLO	287.1 ± 104.1	284.1 ± 79.9	**−1.0**	0.876	303.4 ± 104.6	304.3 ± 90.7	**+0.3**	0.958
VOL	17.6 ± 4.5	16.9 ± 4.1	**−4.0**	0.499	18.7 ± 5.2	17.4 ± 3.4	**−7.0**	0.199
VEL	1.04 ± 0.33	1.04 ± 0.25	**0.0**	0.904	1.14 ± 0.35	1.11 ± 0.31	**−2.6**	0.701

FLO = flow; Nasal PP retina = nasal peripapillary retina; Temporal PP Retina = temporal peripapillary retina; VEL = velocity; VOL = volume.

Hypertension Groups (Mean ± Standard Deviation)

Calculated Ocular Perfusion Pressure	Method of Intraocular Pressure Reduction			Intraocular Pressure After Reduction (mm Hg)	Mean Percentage Reduction of Intraocular Pressure	Mean Number of Weeks Between Reduction of Intraocular Pressure and Second Session of Scanning Laser Doppler Flowmetry
	Medical Therapy	*Argon Laser Trabeculoplasty*	*Surgery (Trabeculectomy)*			
43.2 ± 6.1	4	10	6	14.2 ± 4.7	36.9 ± 16.1	14.2 ± 6.1
42.8 ± 10.6	12	8	0	19.0 ± 2.5	32.7 ± 11.5	14.6 ± 6.3
0.894	—	—	—	0.000	0.349	0.827

increase of 67.2% (P = 0.001). Mean temporal peripapillary retinal flow decreased by 7.4% (P = 0.24), and mean nasal peripapillary retinal flow increased by 0.3% (P = 0.96). In contrast, in the OHT group (Fig 3 and Table 2), none of the flow parameters changed significantly: mean rim blood flow increased by 7.5% (P = 0.41), mean temporal peripapillary flow decreased by 5.2% (P = 0.35), and mean nasal peripapillary flow decreased by 1.0% (P = 0.88).

Large increases in neuroretinal rim flow were measured in the OAG group regardless of the procedure used to reduce IOP. These changes did not always reach statistical significance because of the small sample size in the subgroups. In the medically treated patients (n = 4), the mean increase in rim flow after IOP reduction was 95.0% (P = 0.13), and in the laser-treated patients (n = 10) it was 47.6% (P = 0.07); whereas in the surgically treated patients (n = 6) the mean increase was 85.5% (P = 0.03). Peripapillary retinal flow showed no significant change irrespective of the procedure used ($P \geq 0.22$).

The two groups had disparate mean postreduction IOPs. To examine whether this disparity influenced the results, we matched nine pairs of subjects from the two study groups with identical IOP readings after treatment (Table 3). In these subgroups, OAG patients had a mean (± SD) IOP of 17.89 ± 2.8 after a 23.7% reduction, and OHT patients had a mean IOP of 17.89 ± 2.9 after a 28.3% reduction of IOP. In the OAG subgroup, mean rim flow increased by 54.4% (from 173.8 ± 65.6 to 268.3 ± 121.6, P = 0.061). Mean temporal peripapillary retinal flow decreased by 6.7% (from 287.3 ± 75.8 to 268.0 ± 63.0, P = 0.49), and mean nasal peripapillary retinal flow increased by 5.7% (from 257.2 ± 99.3 to 271.9 ± 97.5, P = 0.47). Conversely, the OHT patients showed no significant change in either the mean rim flow, which decreased by 2.6% (from 350.2 ± 203.3 to 341.1 ± 166.8, P = 0.83), or the temporal or nasal peripapillary retinal flow (−5.9%, P = 0.49 and −12.3%, P = 0.26, respectively). Therefore, we could not find evidence that the postreduction IOP alone influenced the observed difference between the two groups.

We also examined the OAG group for the influence of achieving single-digit postreduction IOP on neuroretinal rim and peripapillary retinal blood flow. A comparison between patients with lower IOP (<10 mmHg) and those with higher IOP (>10 mmHg) was performed. OAG patients with postreduction IOP of <10 mmHg (n = 6) showed an increase in rim flow of 65.6% (from 190.5 ± 86.0 to 315.4 ± 153.1, P = 0.06). Statistical significance was not achieved because of the small size of this group. Peripapillary temporal and nasal retinal flow showed no significant change (−9.4%, P = 0.39; and −1.1%, P = 0.92, respectively). Conversely, OAG patients with postreduction IOP of >10 mmHg (n = 14) showed an increase in rim flow of 68.2% (from 143.8 ± 74.4 to 241.9 ± 112.7, P = 0.007). Peripapillary temporal and nasal retinal flow showed no significant change (−6.5%, P = 0.42 and +1.1%, P = 0.88, respectively). Therefore, increases in neuroretinal rim blood flow were not restricted to patients showing lower IOPs attained after therapy.

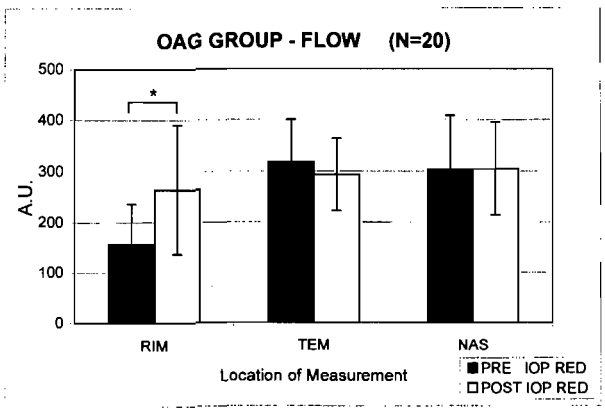

Figure 2. Scanning laser Doppler flowmetry measurements for the parameter flow in the open-angle glaucoma group before and after therapeutic intraocular pressure reduction. (*) P = 0.001, two-tailed distribution paired t test. RIM = neuroretinal rim; TEM = temporal peripapillary retina; NAS = nasal peripapillary retina; PRE IOP RED = blood flow before reduction of intraocular pressure; POST IOP RED = blood flow after reduction of intraocular pressure; A.U. = arbitrary units.

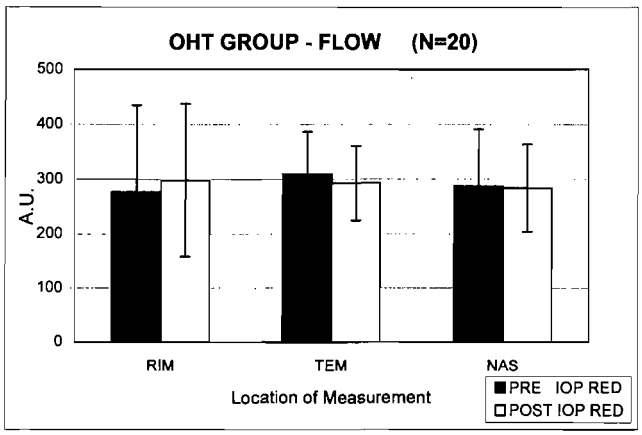

Figure 3. Scanning laser Doppler flowmetry measurements for the parameter flow in the ocular hypertension group before and after therapeutic intraocular pressure reduction. RIM = neuroretinal rim; TEM = temporal peripapillary retina; NAS = nasal peripapillary retina; PRE IOP RED = blood flow before reduction of intraocular pressure; POST IOP RED = blood flow after reduction of intraocular pressure; A.U .= arbitrary units.

Table 3. Characteristics of Post Intraocular Pressure

	Age (Yrs)	Gender (M/F)	Intraocular Pressure before Reduction (mm Hg)	Maximum Recorded Intraocular Pressure (mmHg)	Cup/Disc Ratio	Mean Defect	Error of Refraction
OAG eyes (n = 9)	66 ± 12	5/4	23.7 ± 4.4	30.0 ± 7.8	0.66 ± 0.2	−3.99 ± 7.9	+0.03 ± 1.4
OHT eyes (n = 9)	59 ± 11	4/5	28.3 ± 4.7	30.0 ± 3.2	0.43 ± 0.2	−0.14 ± 3.2	−0.06 ± 2.6
t Test	0.226	—	0.046	0.798	0.035	0.020	0.933

OAG = open angle glaucoma; OHT = ocular hypertension.

There was a significant difference in age between our two study groups ($P = 0.011$, Table 1). To examine the influence of age on our results we compared two age-matched subgroups of 15 OAG and 15 OHT patients (Table 4). In these subgroups, OAG patients had a mean (± SD) age of 63 ± 10 years, whereas OHT patients had a mean age of 62 ± 10 years. After therapeutic IOP reduction, the OAG subgroup showed an 83% increase in mean rim flow ($P = 0.002$), whereas there was only 2% increase in mean rim flow in the OHT subgroup ($P = 0.3$). Mean peripapillary retinal flow had small nonsignificant changes in either group. Thus, age differences between the OAG group and OHT group were found to have no impact on group comparisons and did not seem to affect our perfusion results.

Changes in mean rim height after therapeutic IOP reduction were evaluated in each of the two study groups to determine to what degree such changes may have contributed to measured variations in perfusion data. Changes in the mean rim Z coordinate as defined in Methods are shown in Table 5. After IOP reduction, there was a mean posterior displacement of 7 μm in the neuroretinal rim surface relative to the peripapillary retina in the OAG group ($P = 0.60$). In the OHT group there was a mean anterior displacement of 2 μm in the neuroretinal rim surface relative to the peripapillary retina ($P = 0.85$). Considering that the SLDF is reported to measure flow to a depth of at least 300 μm, it is unlikely that these displacements had a significant impact on our perfusion results.

Discussion

The introduction of SLDF during the last few years has greatly improved our ability to noninvasively assess the hemodynamics of the optic nerve in glaucoma patients. Studies with SLDF have determined that blood flow in the ONH and peripapillary retina is diminished in OAG patients compared with normal subjects[36,37] or OHT patients[38] and that this decrease occurs in patterns consistent with glaucomatous damage. However, there are few published reports on the association between ONH perfusion changes and IOP reduction in patients who require a lower IOP according to clinical evaluation.

Our results indicate that for a very similar IOP reduction (37% versus 33%), OAG patients had far greater improvements in ONH blood flow than did OHT patients (67.2% versus 7.51%). Conversely, peripapillary retinal blood flow, expected to be affected less in glaucoma and serving as an internal control, remained stable in both groups. The difference between the two groups did not seem to be related to the OAG group achieving lower IOPs than the OHT group, nor to the six glaucoma patients achieving single-digit IOPs. Nor did the difference between the two groups depend on the use of filtration surgery: far larger increases in rim blood flow were seen in the OAG group than in the OHT group even when OAG patients undergoing filtration surgery were excluded.

Numerous studies have reported measurements of blood flow in the ONH, the retina, the choroid, and the retrobulbar vasculature in humans and other species. Multiple techniques have been used in both humans and animals such as fluorescein angiography,[39–44] color Doppler imaging,[45–52] pulsatile ocular blood flow,[53–57] laser Doppler flowmetry,[59–61] and SLDF.[36–38] These studies indicate defective ocular perfusion in glaucoma patients compared with OHT and normal subjects. We have recently reported no statistical difference in the mean SLDF perfusion values between OHT patients and normal subjects (Bizzarro et al, Invest Ophthalmol Vis Sci 2001;42:S21). Our current results report that compared with OHT patients, OAG patients initially demonstrated significantly lower perfusion values in the neuroretinal rim but not in the peripapillary retina.

Table 4. Characteristics of Age-Matched

	Age (Yrs)	Gender (M/F)	Intraocular Pressure before Reduction (mm Hg)	Maximum Recorded Intraocular Pressure (mm Hg)	Cup/Disc Ratio	Mean Defect	Error of Refraction
OAG eyes (n = 15)	63 ± 10	7/8	21.7 ± 4.2	28.9 ± 6.6	0.75 ± 0.2	−9.50 ± 8.6	−1.5 ± 2.8
OHT eyes (n = 15)	62 ± 10	7/8	28.7 ± 3.9	29.8 ± 3.5	0.36 ± 0.2	−0.54 ± 2.7	+1.0 ± 0.5
t Test	0.707	—	0.000	0.520	0.000	0.001	0.011

OAG = open angle glaucoma; OHT = ocular hypertension.

Matched Subgroups (Mean ± Standard Deviation)

Calculated Ocular Perfusion Pressure	Method of Intraocular Pressure Reduction			Intraocular Pressure After Reduction (mm Hg)	Mean Percentage Reduction of Intraocular Pressure	Mean Number of Weeks Between Reduction of Intraocular Pressure and Second Session of Scanning Laser Doppler Flowmetry
	Medical Therapy	*Argon Laser Trabeculoplasty*	*Surgery (Trabeculectomy)*			
40.4 ± 6.3	4	6	5	17.9 ± 2.8	25.5 ± 6.17	10.8 ± 5.9
43.7 ± 12.3	9	6	0	17.9 ± 2.9	35.5 ± 13.2	17.1 ± 6.1
0.486	—	—	—	1.000	0.056	0.042

Investigators have also reported improved ocular perfusion after IOP reduction in glaucoma patients. Color Doppler imaging demonstrated significant improvements in retrobulbar hemodynamics after trabeculectomy in patients with chronic glaucoma.[48] Pulsatile ocular blood flow measurements similarly demonstrated a significant increase (29%) in ocular blood flow after reduction of IOP by trabeculectomy.[57] In contrast, a recent study using laser speckle flowgraphy showed little change in the superficial ONH circulation after trabeculectomy in a Japanese population of glaucoma patients.[58]

ONH and retinal perfusion have also been evaluated in OAG and OHT patients after use of topical antiglaucoma therapy. The ability of such medications to alter ocular perfusion has been reported by different authors using different methods to assess ocular blood flow.[62–71] We observed the largest increase in ONH blood flow in our OAG patients receiving medical therapy, but there were too few patients receiving any particular class of drug to look for the impact of individual drugs on ocular blood flow. However, the observed increase in ONH blood flow in the OAG group could not be attributed to medical therapy per se, because increased perfusion was observed regardless of whether medical, laser, or surgical therapy was used.

Blood flow responses to an induced change in IOP using a suction cup have also been studied in animal models[6,72] and in man[73–75] using fluorescein angiography,[76,77] color Doppler imaging,[47,78] and SLDF.[79] Suction-induced IOP elevations reduced retrobulbar, retinal, and ONH perfusion parameters in normal and glaucomatous eyes. Such hemodynamic changes were reversed after normalization of the IOP.

What makes our findings unique is that we believe they are the first quantitative measurements of perfusion obtained directly from the neuroretinal rim tissue of OAG and OHT patients before and after therapeutic IOP reduction. The inclusion of an OHT comparison group, the members of which had not yet manifested full-fledged glaucoma, is also unique.

Our study design and analysis included numerous controls that permit an increased confidence in the results:

1. The immediately adjacent peripapillary tissue shows almost no change in perfusion measurements. This fact excludes the possibility that media opacities or ocular optical changes related to pressure reduction, surgery, or the passage of time contributed artifactually to the increases observed, because such increases would have been observed in both tissues. Changes in Doppler values seen in the OAG group are not seen in the OHT group despite almost identical percentage reductions in IOP, suggesting a physiologic difference between the two groups.
2. Changes in focus might artifactually change SLDF measurements. In recent studies that we have confirmed in our laboratory (data not shown) (Lundmark et al, Invest Ophthalmol Vis Sci 1996 ;37:S265, and Segawa et al, Invest Ophthalmol Vis Sci 1997;38: S774), it was shown that as the focal plane is moved either anterior or posterior to the surface of rim tissue, measured values artifactually increase. We have therefore meticulously established the optimal focal plane during each photography session using the tomography capability of the combined flowmeter/tomograph.
3. We also retrospectively evaluated the position of the surface of the neuroretinal rim with respect to the dominant focal plane, the surface of the peripapillary

Subgroups (Mean ± Standard Deviation)

Calculated Ocular Perfusion Pressure	Method of Intraocular Pressure Reduction			Intraocular Pressure After Reduction (mm Hg)	Mean Percentage Reduction of Intraocular Pressure	Mean Number of Weeks Between Reduction of Intraocular Pressure and Second Session of Scanning Laser Doppler Flowmetry
	Medical Therapy	*Argon Laser Trabeculoplasty*	*Surgery (Trabeculectomy)*			
43.2 ± 6.4	2	7	0	14.1 ± 4.5	35.8 ± 15.8	13.7 ± 6.3
41.2 ± 10.8	4	5	0	19.3 ± 2.7	31.7 ± 11.7	15.1 ± 6.4
0.556	—	—	—	0.001	0.425	0.528

Table 5. Mean Rim Z Coordinate Change Relative to Reference Plane (Mean ± Standard Deviation)

Group	Mean Rim Z Coordinate Before Intraocular Pressure Reduction (mm)	Mean Rim Z Coordinate After Intraocular Pressure Reduction (mm)	Change (mm)	P Value
OAG (n = 20)	0.104 ± 0.09	0.111 ± 0.06	+0.007	0.60
OHT (n = 20)	0.064 ± 0.12	0.062 ± 0.12	−0.002	0.85

OAG = open angle glaucoma; OHT = ocular hypertension.

retina. This analysis demonstrates that after IOP reduction, only minimal displacements of several micrometers were observed in each group. These shifts in the mean rim Z coordinate with respect to the focal plane are too small to have artifactually contributed to the observed changes in perfusion values.

4. When patients were matched between the two groups for their IOP after therapy, an improvement in rim blood flow was observed only in the OAG group. As well, within the OAG group, patients with single-digit postreduction IOPs had the same magnitude increases in rim blood flow as did those patients achieving higher pressures.
5. Incisional surgery was performed on six OAG patients but none of the OHT patients. Among the 14 OAG patients undergoing medical or laser therapy, a mean 63.2% ($P = 0.012$) improvement of rim flow was recorded, suggesting that the effect observed for the entire group was not related to incisional surgery per se.
6. When patients were matched between the two groups for age, the OAG subgroup maintained the same significant increase in mean rim flow, whereas there was no change in mean rim flow in the OHT subgroup.

The observed changes provide compelling evidence consistent with the hypothesis concerning defective autoregulation of the ONH blood flow in glaucoma. In the OHT group, mean rim blood flow did not change significantly in response to a 33% reduction in IOP, suggesting that these patients have intact ONH autoregulation. In contrast, the OAG group demonstrated an increase in the mean rim blood flow of 67% ($P = 0.001$) in response to a 37% IOP reduction, suggesting that these patients have defective ONH autoregulation. Peripapillary retinal blood flow changes were not significant in OHT and OAG patients, suggesting an intact retinal autoregulation in both groups.

Because a small subset of OHT patients eventually develop glaucoma, and based on the assumption that a vascular disturbance contributes to the development of glaucomatous optic neuropathy, it might be expected that some of our OHT patients would show significant improvements in neuroretinal rim blood flow after therapeutic IOP reduction. In fact 4 of the 20 OHT patients (20%) showed an improvement in rim blood flow exceeding 25% after IOP reduction. Thus, a small subset of the OHT patients may be demonstrating defective autoregulation, although as a group their blood flow showed no significant change.

Although the superficial ONH and the peripapillary retina are considered to be both perfused by the central retinal artery, the autoregulation of this flow at the level of the microvasculature may differ between both tissues. This would not be surprising given that the neuroretinal rim is highly abnormal in glaucoma, whereas the peripapillary retina, below the level of the nerve fiber layer, is often (but not always) well preserved. As well, although it is assumed that SLDF does not penetrate beyond 300 μm,[20,21] this assumption has not been rigorously verified. Finally, the superficial neuroretinal rim may be receiving a significant contribution to its perfusion by deeper vasculature whose autoregulatory control differs from that of the retina.

The data also indicate that improvement of ONH perfusion in OAG patients may be part of the beneficial response to ocular hypotensive therapy, whether medical, laser, or surgery. This concept has been supported by other studies.[48,57]

Further research into the role of ONH blood flow in the different forms of glaucoma, the impact of autoregulation, and the effect of diverse therapies on ocular blood flow should lead to improved understanding of the disease and better therapy for glaucoma patients.

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Correlation Between Finger Blood Flow and Changes in Optic Nerve Head Blood Flow Following Therapeutic Intraocular Pressure Reduction

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Purpose: To correlate finger blood flow and changes in optic nerve head (ONH) blood flow following therapeutic intraocular pressure (IOP) reduction in open-angle glaucoma (OAG) and ocular hypertension (OHT).

Methods: Seventeen open-angle glaucoma patients and nineteen ocular hypertension patients underwent therapeutic IOP reduction followed by a minimum of 4 weeks of follow-up. Optic nerve head blood flow measurements were obtained by scanning laser Doppler flowmetry using full-field perfusion image analysis. Finger blood flow was measured using the Transonic laser Doppler Flowmeter. Finger blood flow was measured at baseline, after immersion in warm water (40°C) for 2 minutes (Flow Max), and after immersion in cold water (4°C) for 10 seconds (Flow Min). Patients were identified as vasospastic if their Flow Max/Flow Min >7. Statistical comparisons were performed using two-tailed distribution paired T-test and Pearson's correlation factor.

Results: For similar mean percentage intraocular pressure reduction, vasospastic patients had greater improvements in rim blood flow than did non-vasospastic patients [+35% versus +13%] ($P = 0.01$). While there was no difference in rim blood flow changes in the vasospastic versus the non-vasospastic OAG group, the vasospastic ocular hypertension group showed 18% increase in rim blood flow whereas the non-vasospastic ocular hypertension group showed an 8% decrease. A significant negative correlation was also found in the open-angle glaucoma group between rim blood flow change and Flow Max (-0.681, $P = 0.003$). In contrast, no such correlation was found in the ocular hypertension group (+0.144, $P = 0.556$).

Conclusion: OAG patients had a significant negative correlation between changes in rim blood flow and maximum finger Doppler flow. Among ocular hypertension patients, increased rim blood flow was only found in the vasospastic group, though this increase was not statistically significant. These results suggest that open-angle glaucoma and ocular hypertension patients with the most severe vasospastic disease may show the greatest improvements in rim blood flow after sustained intraocular pressure reduction.

Key Words: Autoregulation, glaucoma, optic nerve head blood flow, vasospasm

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INTRODUCTION

The mechanism of damage to the optic nerve head (ONH) in open-angle glaucoma (OAG) is almost certainly multifactorial.[1] Elevated intraocular pressure (IOP) remains the risk factor most commonly associated with glaucomatous optic neuropathy. However, numerous other variables involved in the development and progression of OAG have been identified.[2–7] Vascular risk factors in particular have been extensively studied.[8,9] These include systemic blood pressure alterations,[10–12] diabetes,[13,14] reduced ocular blood flow,[15–18] and vasospasm.[19–24] Although such vascular risk factors have been postulated several decades ago, only recent technical advances have enabled research into the associated microcirculatory anomalies and their impact on blood flow autoregulation.

Vasospasm is reported as an inappropriate constriction of the smooth muscles of the microcirculation with no recognizable anatomic alterations.[25] It can involve different organs simultaneously or successively. Vasospasm is an important factor in the pathogenesis of several diseases such as migraine, Raynaud's syndrome, variant angina, and normal tension glaucoma. It can occur in healthy subjects in response to diverse stimuli including exposure to cold, nicotine, or emotional stress as well as in association with a variety of diseases, including autoimmune and infectious diseases.[26] Although such vasospasm normally leads to reversible functional damage, it may rarely lead to irreversible ischemic changes.[27]

Phelps and Corbett in 1985[28] were the first to suggest the possible role of vasospastic phenomena in the development and progression of glaucomatous optic neuropathy. They

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The authors have no proprietary interest in the Heidelberg Retina Flowmeter, the SLDF analysis software version 3.3, or the Transonic laser Doppler flowmeter.

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found that 47% of their patients with normal tension glaucoma also suffered from migraine. They reported that such frequent occurrence of migraine, Raynaud's syndrome, and variant angina suggests generalized vasospastic phenomena. Gasser et al in 1987[19] described ocular vasospasm in which patients with unexplained scotomas had abnormal capillaroscopic response to cold in the nailfold of the fingers. The scotomas were aggravated by the immersion of a hand in cold water and improved after administration of calcium channel blockers.[29] Gasser et al assumed that patients with tendency to vasospasm exhibit ocular vascular reactions similar to those that occur in the capillaries of the fingers. In 1988, Guthauser et al[22] demonstrated a statistically significant relationship between patient's history of cold hands and the outcome of both the visual field cold water test and the nailfold capillaroscopic test. The visual field results were also found to correlate significantly with the capillaroscopic results. In 1988, Drance et al,[24] using Doppler blood-flow measurements in the finger and a cold test, showed that in non-glaucomatous subjects, 26% without migraine had a positive vasospastic response whereas 64% with classic migraine showed such a response. Of the patients with low-tension glaucoma, 65% showed a positive vasospastic response. The Collaborative Normal Tension Glaucoma Study[30] demonstrated a statistically significant increased risk of progression in glaucoma patients suffering from migraine. The relationship between vasospastic changes and structural ischemic changes in glaucoma is still not well understood, though it has been repeatedly suggested in the literature that insufficient vasospastic regulation in the ONH could explain the abnormal or defective autoregulation of ONH blood flow.[31,32]

In a recent study, we have demonstrated a significant improvement (67%, $P = 0.001$) in ONH blood flow in OAG patients after therapeutic IOP reduction whereas patients with ocular hypertension (OHT) did not demonstrate such a change. We attributed the observed changes to defective autoregulation of the ONH blood flow in glaucoma.[33] In the present study we extend our findings by examining whether vasospastic OAG and OHT patients demonstrate different ONH blood flow changes in response to therapeutic reduction of IOP when compared with non-vasospastic patients. We also examine the correlation between finger blood flow and changes in ONH blood flow after therapeutic IOP reduction in OAG and OHT.

PATIENTS AND METHODS

Seventeen patients with OAG and 19 patients with OHT with clinical indication for therapeutic IOP reduction were included in this study. These patients were a subset of a previous study population.[33] All patients had a minimum of 20% IOP reduction after medical, laser, or surgical intervention as well as a minimum of 4 weeks of follow-up.

Patients with OAG had glaucomatous optic neuropathy, characteristic nerve fiber bundle visual field defects, and gonioscopically open angles with no restrictions for IOP. Ocular hypertensives had a history of repeated IOPs greater than 24 mm Hg with normal visual fields and normal or suspect ONH appearance. A detailed medical and ophthalmic history was obtained from all patients, including a questionnaire addressing complaints of cold hands and feet and migraine as well as cardiac problems, intermittent claudication, smoking, alcohol and caffeine intake, and exposure to stress.

Optic nerve head blood flow measurements were obtained by scanning laser Doppler flowmetry (SLDF) using Heidelberg Retina Flowmeter images (HRF, Heidelberg Engineering, Heidelberg, Germany). The SLDF is a noninvasive instrument combining both a laser Doppler flowmeter with a scanning laser technique. Technical details related to the instrument are discussed elsewhere.[34] SLDF measures the amount of backscattered light from the ONH and peripapillary retina. A 2-dimensional map of microvascular perfusion of the area to be studied (2.7 mm × 0.7 mm) is thus generated.

Scanning laser Doppler flowmetry imaging of the ONH was performed at baseline then at a minimum of 1 month after sustained IOP reduction as previously described.[33] Automatic full-field perfusion image analysis[35] was then performed on each of the HRF images and mean values for flow in arbitrary units (au) were obtained from 5 perfusion images. Our method of SLDF imaging for ONH perfusion as well as its intrasession and inter-session reproducibility values have been described in detail in previous publications.[36]

Finger blood flow measurements were obtained by the Transonic Laser Doppler Flowmeter (Transonic Systems Inc., Ithaca, NY). This device uses a low-intensity laser beam transmitted through a fiber optic cable to illuminate the nail-fold capillaries in the finger. A receiver detects light reflected by stationary structures (such as tissue) and moving particles (mainly red blood cells). The latter portion of reflected light undergoes a Doppler frequency shift allowing computation of the proportion of the flow due to red blood cells. Baseline flow was measured on the underside of the end of the middle finger of a randomly selected hand. After a stable flow reading was obtained the hand was immersed in warm water (40°C) for 2 minutes (Fmax). The hand was then immersed in ice-cold water (4°C) for 10 seconds (Fmin) and then finally placed at room temperature for 10 minutes (recovery period) (Fig. 1). Finger flow measurements were made continuously by the laser Doppler flowmeter and transmitted in real-time to a computer via an interface. Blood pressure measurements were taken when recording flow at baseline, exposure to warmth, and recovery. Patients were classified into vasospastic and non-vasospastic groups. A vasospastic response was taken as present when the vasospasticity index (the ratio of maximum flow to minimum flow) exceeded 7 (ie, Fmax/Fmin >7). The methodology used for finger blood flow measurements as well as the definition of a positive vasospastic response were both based upon a leading study by Drance et al.[24]

Changes in ONH and peripapillary retinal blood flow were evaluated using 2-tailed distribution paired t test whereas group differences were evaluated using one-way analysis of variance (ANOVA). Correlation between changes in ocular blood flow and finger blood flow were performed on each of the OAG and OHT groups using Pearson linear correlation factor. Statistical significance was set at $P < 0.05$.

RESULTS

Data for both ocular and finger blood flow was obtained from 36 patients, 17 OAG patients and 19 OHT patients.

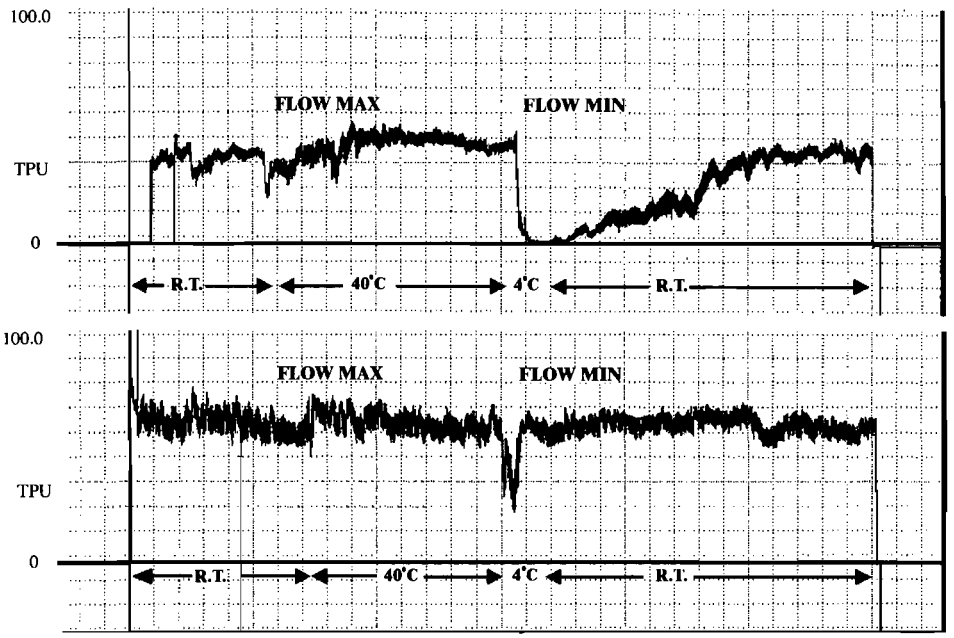

FIGURE 1. Top: Tracing of peripheral blood flow in a vasospastic patient showing a low baseline flow at room temperature (R.T) with a small increase after immersion of the hand in warm water (40°C), during which time Flow Max is determined. A marked decrease in flow occurred after immersion of the hand in cold water (4°C) with a delayed recovery to baseline. Bottom: Tracing of peripheral blood flow in a nonvasospastic patient showing a normal baseline flow at room temperature (R.T) with no increase after immersion of the hand in warm water (40°C). The flow then decreased after immersion of the hand in cold water (4°C) (but less markedly than in the top tracing) with a rapid recovery to baseline. Markings on x-axis are 15 seconds. TPU: tissue perfusion units.

Based on their vasospastic response (ratio of maximum flow to minimum flow exceeding 7 (ie, Fmax/Fmin >7), patients were classified into vasospastic and non-vasospastic groups. There were 22 patients in the vasospastic group (10 patients with OAG and 12 patients with OHT) and 14 non-vasospastic groups (7 patients with OAG and 7 patients with OHT).

Patient characteristics in the vasospastic and non-vasospastic groups are shown in Table 1. The mean age (±SD) was 58.7 ± 12.5 years for the vasospastic group (range 42–70 years) and 67.7 ± 8.4 years for the non-vasospastic group (range 52–79 years), [$P = 0.01$, Student t test].

The baseline rim blood flow and temporal and nasal peripapillary retinal blood flow were analyzed with a 2-way ANOVA. For rim blood flow, the interaction between vasospastic (or not) and diagnosis (OHT and OAG) was not significant [$F(1,32) = 3.446$, $P = 0.073$]. Baseline rim blood flow in vasospastic patients was 187.7 ± 96.8 au whereas in non-vasospastic patients it was 281.7 ± 181.7 au [$F(1,32) = 6.336$, $P = 0.017$]. Baseline rim blood flow in OAG patients was 153.9 ± 83.0 au whereas in OHT patients it was 287.2 ± 154.9 [$F(1,32) = 15.593$, $P < 0.001$]. This analysis indicates that independent of diagnosis there was a significant difference between baseline rim flow values in vasospastic versus non-vasospastic patients. For baseline temporal and nasal peripapillary retinal blood flow there were no significant interactions or mean differences between groups (all P values are greater than 0.5).

Following sustained therapeutic IOP reduction of 32%, the vasospastic group (Table 2) showed a significant improvement in mean rim blood flow of 34.5% [$P = 0.01$] whereas mean peripapillary retinal flow showed no significant change [$P \geq 0.18$] (2-tailed paired t test). In contrast, in the non-vasospastic group and following sustained therapeutic IOP reduction of 37% (Table 2), mean rim blood flow increased by 13.3% [$P = 0.32$] whereas mean peripapillary retinal flow showed no significant change [$P \geq 0.27$] (2-tailed paired t test).

Among the OAG patients, and following a similar % IOP reduction of 36%, vasospastic patients showed an increase in mean rim blood flow of 64.8% [$P = 0.003$] whereas non-vasospastic patients showed an increase of 62.3% [$P = 0.09$] (2-tailed paired t test) (Table 2). On the other hand, among the OHT patients, vasospastic patients showed an increase in mean rim blood flow of 18.4% [$P = 0.27$] whereas non-vasospastic

TABLE 1. Patient Characteristics (mean ± SD) in Vasospastic Group and Non-Vasospastic Group

	Age Yrs	Sex M/F	IOP Pre	IOP Max	C/D Ratio	M.D.	Refr. Error	OPP	IOP Post	% Red	Dur. Wks
Vasospastic group N = 22	59 ± 13	9/13	25 ± 5	33 ± 5	0.6 ± 0.3	−4.6 ± 7.6	−0.8 ± 3.3	44 ± 8	17 ± 4	32 ± 12	14 ± 5
Non-vasospastic group N = 14	68 ± 8	8/6	27 ± 6	31 ± 5	0.6 ± 0.3	−4.4 ± 7.6	+0.6 ± 1.9	40 ± 9	17 ± 5	37 ± 14	17 ± 7
T Test	0.01		0.35	0.26	0.70	0.94	0.12	0.14	0.97	0.33	0.24

IOP Pre, intraocular pressure prior to reduction (mm Hg); IOP Max, maximum recorded intraocular pressure (mm Hg); C/D, cup/disc ratio, M.D, mean defect of visual field; Refr. Error, error of refraction; OPP, calculated ocular perfusion pressure; IOP Post, intraocular pressure after reduction (mm Hg); % Red., Mean percentage reduction of IOP; Dur. (wks): Mean number of weeks between reduction of IOP and second session of SLDF.

Two-tailed Student's t-test.

TABLE 2. Changes in Neuroretinal Rim Blood Flow in Vasospastic Versus Nonvasospastic Open-Angle Glaucoma and Ocular Hypertensive Patients

	Vasospastic Group N = 22				Non-Vasospastic Group N = 14			
Subjects	Pre IOP Red	Post IOP Red	% Change	*P* Value	Pre IOP Red	Post IOP Red	% Change	*P* Value
OAG + OHT	187.2 ± 96.0	251.7 ± 102.4	+35	0.01	274.7 ± 181.7	311.2 ± 160.3	+13	0.32
OAG	142.3 ± 99.3	234.4 ± 106.5	+65	0.003	169.0 ± 49.8	274.3 ± 137.1	+62	0.09
OHT	224.6 ± 78.6	266.0 ± 101.2	+18	0.27	380.5 ± 207.3	348.0 ± 183.7	−8.5	0.38

OAG, open angle glaucoma; OHT, ocular hypertension; Pre IOP Red, flow values before IOP reduction; Post IOP Red, flow values after sustained IOP reduction; % Change, percentage of change in flow.

patients showed a decrease of 8.5% [$P = 0.38$] (2-tailed paired *t* test) following % IOP reduction of 29% versus 38% (Table 2).

Correlations between the significant changes in ONH blood flow and finger blood flow parameters were then performed on each of the OAG and OHT groups. A significant negative correlation was demonstrated in OAG patients between changes in rim blood flow following sustained therapeutic IOP reduction and Flow Max (maximum finger flow when the hand was immersed in warm water) [$R = -0.681$, $P = 0.003$, Pearson linear correlation factor] (Fig. 2 , top). In contrast, no such correlation was found in OHT patients [$R = +0.144$, $P = 0.556$, Pearson linear correlation factor] (Fig. 2, bottom). Following therapeutic IOP reduction, OAG patients with low Flow Max demonstrated an increase in neuroretinal rim blood flow of 158 au versus 45 au for the high Flow Max group [$P = 0.020$, Student *t* test]. No correlation was shown between the other peripheral vascular parameters (Flow Base, Flow Min, vasospasticity index, or duration until recovery) and neuroretinal rim flow changes in either OAG or OHT patients.

We then analyzed the detailed medical and ophthalmic history obtained from OAG patients as well the data from the questionnaire addressing complaints of cold hands and feet and migraine as well as cardiac problems, intermittent claudication, smoking, alcohol and caffeine intake, and exposure to stress in an attempt to identify the characteristics of OAG patients with low peripheral maximum Doppler flow (Flow Max) as defined by the finger laser Doppler flowmeter results. OAG patients with low "Flow Max" compared with those with high "Flow Max" tended to be somewhat younger in age (63 versus 69 years), showed no difference in the frequency of migraine symptoms, cold hands and feet, smoking, or hypertension, and had a similar maximum-recorded IOP (27.5 versus 27.0 mm Hg). However, they tended to be more vasospastic (vasospasticity index of 12 versus 6 [$P = 0.16$, Student *t* test]) and reported a higher incidence of current or previous emotional stress (7/8 versus 5/9 patients). We concluded that patients with low "Flow Max" tended to have predominantly vasospastic profiles rather than atherosclerotic ones.

DISCUSSION

This study demonstrates that for a similar percentage of therapeutic IOP reduction [32% versus 37%] vasospastic patients show greater improvements in ONH blood flow than do non-vasospastic patients [+35% versus +13%]. In ocular hypertensives, improved ONH blood flow following IOP reduction was shown in vasospastic patients [+18%] but not in non-vasospastic patients [−8%]. Changes in ONH blood flow showed a significant negative correlation with maximum finger blood flow in OAG patients but not in OHT patients, such that glaucoma patients with lower finger blood flow had greater increases in ONH blood flow.

We believe that the reported changes in ONH blood flow correlated more with vasospastic indices rather than atherosclerotic ones. The medical and ophthalmic history obtained from OAG patients as well as the data derived from the conducted questionnaire indicate that OAG patients with low

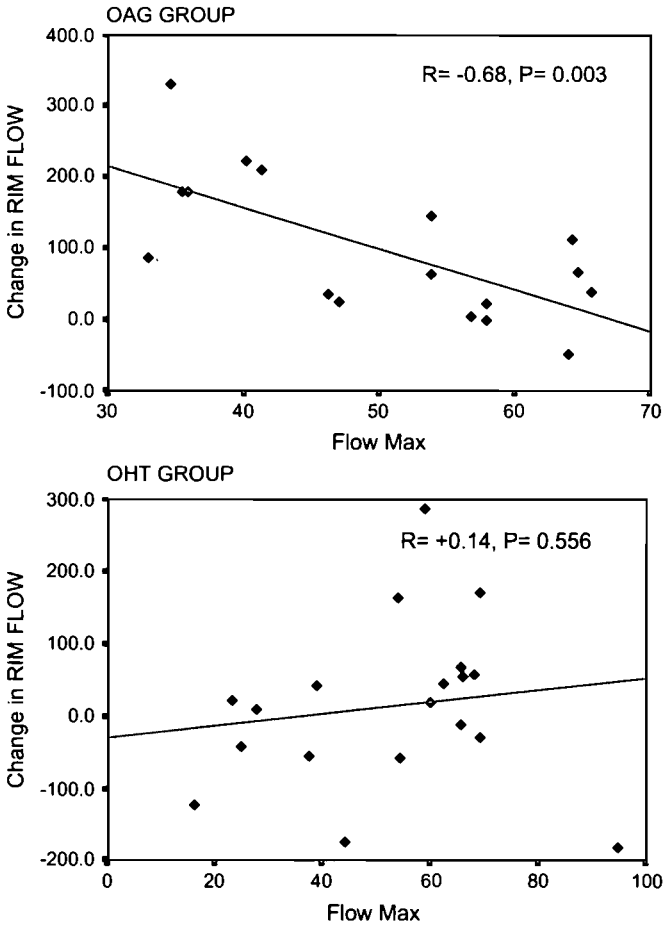

FIGURE 2. Correlation between flow max (maximum finger blood flow) and change in neuroretinal rim blood flow in OAG and OHT patients.

maximum finger blood flow (Flow Max) tended to be vasospastic rather than to have significant atherosclerotic cardiac or peripheral vascular disease. OAG patients with low Flow Max also reported being under a higher incidence of current or previous emotional stress. Similar findings were reported among vasospastic patients by Flammer and Prunte.[37]

Vasospasm is a systemic disorder. Several studies have reported a high prevalence of migraine, Raynaud's disease, and variant angina in patients with peripheral vasospasm.[38] Migraine was diagnosed in 26% and Raynaud's disease in 24% of patients with variant angina.[39] In 11% of the patients with variant angina, migraine and Raynaud's disease occurred together.[39] Furthermore, an association has been reported between variant angina or migraine and vasospasm in the retinal circulation.[40]

Vasospastic patients had significantly lower baseline rim blood flow compared with non-vasospastic patients. We believe that such a difference in baseline ONH blood flow between study groups is related more to shared digital and ocular vasospastic properties than to population differences or instrument variability. Apart from age, which was significantly lower in patients with vasospasm and reflects a decrease of vasospastic tendencies with increasing age, the two study groups show similar and in some cases identical demographic characteristics. Instrument or technique variability has also been comprehensively evaluated in a prior study of the reproducibility of the SLDF technique.[36] In that study we used the automated technique of SLDF full-field perfusion analysis[35] together with obtaining mean values from 5 high-quality perfusion images. We reported an intra-session variation of 16.0% and an inter-session variation of 15.1% in the neuroretinal rim of glaucoma patients.

To the best of our knowledge this is the first report linking peripheral vasospastic disease to changes in ONH blood flow. Previous studies demonstrated that glaucoma patients have significantly increased incidence of peripheral vasospasm.[41,42] Other studies observed that visual fields in some glaucoma patients improve following carbon dioxide inhalation and point to possible involvement of an abnormal vascular regulation.[43] Correlations of ONH appearance with age in vasospastic patients suggest that a functional vasospasm might play an important role in the development of glaucomatous optic neuropathy.[44] In our study, the mean age of patients with vasospasm was significantly lower than patients without vasospasm ($P = 0.01$). A similar finding was reported by Guthauser et al,[22] consistent with the possibility that vasospastic tendencies decrease with age.

Schulzer et al[45] reported that OAG patients could be divided into 2 groups: patients who were predominantly vasospastic and patients who had predominantly small vessel disease. In the vasospastic group there was a high positive correlation between the amount of visual field damage and the highest intraocular pressure. The authors suggested that this group might have "pressure-dependent" glaucoma. Among the OAG patients with small vessel disease, no such correlation was found and the authors suggested that this group might have "pressure-independent" glaucoma. The present study suggests that ONH blood flow is more sensitive to intraocular pressure in OAG and OHT patients that are vasospastic. It is interesting to speculate that this relationship might underlie the findings of Shulzer et al. Similarly, the Collaborative Normal-Tension Glaucoma Study[46] found that whereas women with migraines benefited from IOP lowering, patients with a history of cardiovascular disease or a family history of stroke did not, once again linking vasospasticity, IOP, and progressive visual field loss.

Vasospasm has been observed in the posterior ciliary arteries as well as in the choroidal vessels,[47] and was assumed to negatively influence the circulation in the ONH. It is also possible that such vasospasm may occur directly in the vasculature of the ONH. In a recent study by Hasler and coworkers[31] the authors, using single-point laser Doppler flowmetry, report a significant positive correlation between calculated ocular perfusion pressure and blood flow in the choroid of vasospastic subjects. A similar study by Gherghel et al,[32] using color Doppler imaging, reported a significant positive correlation between calculated ocular perfusion pressure and both peak-systolic velocity and end-diastolic velocity as well as a significant negative correlation with resistivity index in the central retinal artery of vasospastic subjects. Such correlations did not occur in the control group. Both studies conclude that blood flow-regulating mechanisms might be different between vasospastic and non-vasospastic subjects and point to an abnormal vascular regulation in the retrobulbar circulation of vasospastic patients.

The observed changes in optic nerve blood flow following IOP reduction indicate defective optic nerve vascular autoregulation in much of the studied cohort.[33] Defective autoregulation of ONH blood flow has been reported to occur as result of decreased perfusion pressure, increased blood viscosity, or increased local resistance.[48] Increased local resistance is manifested as a reduced vascular diameter and may be produced by mechanical obstruction by thrombosis, embolization, arteriosclerosis, or external compression. Reduced vascular diameter can also be due to a reversible spasm of the smooth muscle cells in the vessel wall. In their analysis of optic nerve blood flow abnormalities in glaucoma, Flammer and Orgul[48] considered arteriosclerosis as a principal factor for the increased vascular resistance that contributes to defective optic nerve perfusion. Although experimental studies by Hayreh[49] indicate that arteriosclerosis might increase the sensitivity to IOP elevations and although some arteriosclerotic patients were shown to present with a sclerotic type of glaucoma,[50] Flammer and Orgul[48] believed there was currently very little evidence linking arteriosclerosis to glaucomatous optic neuropathy. Flammer hypothesized increased local resistance to blood flow as a risk factor in the development and progression of glaucoma. He attributed this resistance to a functional rather than a structural change, namely to an abnormal or defective autoregulation of blood flow. Autoregulation implies the capacity of an organ to regulate its perfusion so as to maintain a constant sufficient metabolic supply despite the change in ocular perfusion pressure. An abnormal or defective autoregulation could be expressed not only as excessive vasoconstriction but also as lack of appropriate vasodilatation.[51,52]

Our results provide evidence consistent with the hypothesis concerning defective autoregulation of the ONH blood flow in glaucoma and its vasospastic origins. OAG patients showed evidence suggesting defective autoregulation of ONH

blood flow and demonstrated a significant negative correlation between changes in ONH blood flow and maximum Doppler flow in the finger. Vasospastic OHT patients also showed a tendency suggestive of defective ONH autoregulation as manifested by an increase, though insignificant, in ONH blood flow following IOP reduction.

Our findings also identify a subgroup of OAG patients with severe vasospastic disease. We believe these patients demonstrate increased local resistance to blood flow as a result of constant vasoconstriction or inappropriate vasodilatation and as manifested by their low Flow Max. These patients were also shown to have the largest improvements in neuroretinal rim blood flow following sustained IOP reduction, which points to an abnormal or defective autoregulation in ONH blood flow. OHT patients did not demonstrate this correlation between Flow Max and ONH blood flow suggesting that their vasospastic disease, when present, may be less pervasive.

In the OHT group, vasospastic patients showed a similar trend of improved ONH blood flow whereas non-vasospastic patients did not. In a clinical context, a small subset of OHT patients eventually develop glaucoma, and based upon the assumption that a vascular disturbance contributes to the development of glaucomatous optic neuropathy, it might be expected that some of our OHT patients would show significant improvements in neuroretinal rim blood flow following therapeutic IOP reduction. In fact, 3 of the 19 OHT patients (16%) showed such an improvement in rim blood flow. Thus, a small subset of the OHT patients may be demonstrating defective autoregulation, although as a group their blood flow showed no significant change. Such a behavior of OHT patients raises the question of whether this factor might identify OHT patients at risk for developing glaucoma.

The relatively small sample size as well as the large variation might have similarly limited our results related to both the OAG and OHT subgroups. However, to the best of our knowledge, we believe the findings of the present study suggest for the first time an abnormal or defective autoregulation occurring in the ONH of vasospastic subjects. It also shows a significant correlation between the presence of lower peripheral blood flow (Flow Max) in OAG patients and improvement in ONH blood flow with therapeutic IOP reduction and thus provides evidence that systemic vasospasm or vascular dysregulation might actually underlie such a defective ONH autoregulation in glaucoma.

We believe our data may form the basis for a larger long-term study to examine the relationship between digital and ocular vasospasm and their impact on autoregulation of ONH blood flow and on progression of glaucomatous optic atrophy in OAG and OHT. We believe that such studies will eventually lead to a better understanding of the disease process as well as to novel therapeutic approaches for our patients.

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Relationship Between Central Corneal Thickness and Changes of Optic Nerve Head Topography and Blood Flow After Intraocular Pressure Reduction in Open-angle Glaucoma and Ocular Hypertension

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Objectives: To investigate changes in optic nerve head topography and blood flow after therapeutic intraocular pressure reduction and to correlate them with central corneal thickness.

Methods: Sixteen patients with open-angle glaucoma and 16 patients with ocular hypertension underwent Heidelberg retina tomography and scanning laser Doppler flowmetry in 1 eye before and at least 2 months after a mean 35% sustained therapeutic reduction in intraocular pressure. Patients were assigned to a thin or thick group based on their median central corneal thickness.

Results: Compared with 16 patients with thick corneas (mean±SD central corneal thickness, 587±31 μm), the 16 patients with thin corneas (518±32 μm) had greater reductions in mean (36±32 vs 4±36 μm, $P=.003$) and in maximum cup depth (73±107 vs 4±89 μm, $P=.02$). These changes were not statistically significantly different between the patients with open-angle glaucoma and those with ocular hypertension. Smaller mean±SD improvements in neuroretinal rim blood flow were seen in patients with thinner corneas compared with those with thicker corneas (35±80 vs 110±111 arbitrary units, $P=.04$).

Conclusion: Patients with open-angle glaucoma and ocular hypertension with thinner corneas show significantly greater shallowing of the cup, a surrogate marker for lamina cribrosa displacement (compliance), and smaller improvements of neuroretinal rim blood flow after intraocular pressure reduction.

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ALTERED LAMINA CRIBROSA compliance has long been postulated to have a role in the development of open-angle glaucoma (OAG). Lamina cribrosa mobility has been studied in ex vivo human[1-6] and monkey[7,8] eyes, in living human[9-13] and monkey[14,15] eyes, and in histological studies.[1,8,16-18] Findings from some studies[8,15,19] suggest that there may be an initial hypercompliance in early glaucoma followed by reduced compliance (ie, increased rigidity) later in the course of the disease. In most patients with glaucoma, the central lamina cribrosa is covered by little or no neural or glial tissue. Therefore, lamina cribrosa compliance can be readily estimated using scanning confocal laser tomography by examining the position of the base of the cup relative to the retinal surface after intraocular pressure (IOP) changes.[9,11-13]

Considerable evidence suggests that abnormal optic nerve blood flow has a role in the development of glaucomatous optic neuropathy.[20] Recent data indicate that optic nerve head neuroretinal rim blood flow improves significantly in patients with OAG after sustained therapeutic IOP reduction.[21] Among patients with ocular hypertension (OHT), such improvements were limited to vasospastic subjects.[22] The prognostic significance of these blood flow changes remains to be determined.

Findings suggest that the presence of a thin cornea is linked to the development of glaucoma among patients with OHT,[23] as well as to the severity of OHT[24,25] and OAG.[26,27] In OHT and OAG, a thin cornea is more strongly associated with disease severity than IOP.[23,27] Underestimated Goldmann tonometric pressures seem to only partly explain the relationship between thin corneas and increased glaucoma risk. The other mechanisms underlying this relationship are unknown. Corneal thickness has been linked to scleral thickness.[28-30] In this study, we examined the relationship between central corneal thickness and lamina cribrosa compliance. Because the blood vessels that feed the optic nerve head run through the lamina cribrosa, we also examined changes

in neuroretinal rim blood flow that occur with IOP-dependent lamina changes.

METHODS

The study protocol was approved by the Ethics Committee of Maisonneuve-Rosemont Hospital, Montreal, Quebec, and all patients signed an informed consent form. Patients with OAG had gonioscopically confirmed open angles and manifested at least 2 of the following 3 criteria: characteristic nerve fiber bundle visual field defects, glaucomatous optic neuropathy, and a history of IOP greater than 21 mm Hg. Patients with OHT had a history of IOP greater than 24 mm Hg on at least 2 occasions, normal visual fields, and normal or suspect optic nerve head appearance based on slitlamp biomicroscopy. Subjects were excluded if any abnormal ocular findings were present other than pseudophakia, if significant media opacities precluded scanning laser Doppler flowmetry (SLDF) imaging, and if they were unable to comply with the study protocol.

Medical and ocular histories were recorded, and IOP, refractive errors, and best-corrected visual acuity were measured before the baseline study visit. A basic ophthalmologic examination, including biomicroscopy, ophthalmoscopy, and gonioscopy, was performed, and the visual field was assessed using automated perimetry (Humphrey Field Analyzer, program 24-2; Humphrey Instruments, San Leandro, Calif).

Thirty-two patients having clinical indications for IOP reduction were recruited from the hospital glaucoma clinics and underwent confocal scanning laser tomography with the Heidelberg retina tomograph (version 2.01; Heidelberg Engineering, Heidelberg, Germany) and SLDF of the optic nerve head with the Heidelberg retina flowmeter and SLDF software version 3.3 (Heidelberg Engineering)[31] before and at least 2 months after a minimum IOP reduction of 20%. For Heidelberg retina tomographic imaging, the mean topographies were derived from 3 high-quality images. The SLDF values for neuroretinal rim blood flow were derived from the mean of 5 high-quality images as described previously.[21]

As indicated clinically, IOP was reduced using topical hypotensive medications, argon laser trabeculoplasty, or filtration surgery. All patients were treated by one of us (M.R.L.). One eye was studied in each patient. If both eyes required therapy, the eye with the clearest media was chosen. We used the Heidelberg retina tomography stereometric variables of mean cup depth and maximum cup depth to estimate lamina cribrosa position in micrometers before and after IOP reduction. We used the SLDF variable of flow in all pixels overlying the neuroretinal rim to determine neuroretinal rim blood flow in arbitrary units before and after IOP reduction. Central corneal pachymetry was determined using an ultrasound pachymeter (DGH 500 Pachette; DGH Technology, Fraser, Pa) using the mean of the 3 closest of 5 consecutive measurements. Values are given as mean±SD. Statistical evaluations were performed using Pearson product moment correlation test and *t* test. Statistical significance was set at $P<.05$. Analysis of covariance (ANCOVA) was used to control for covariables.

RESULTS

Patient characteristics are given in **Table 1**. There were 16 patients with OAG and 16 patients with OHT. Patients were assigned to the thin group or to the thick group based on their median central corneal thickness (CCT). To keep the groups balanced with respect to diagnosis, the 8 patients with OAG with the thinnest corneas were grouped with the 8 patients with OHT with the thinnest corneas to form the thin group. Clinical variables other than CCT did not differ significantly between the thin (n=16) and thick (n=16) groups.

The optic nerve head stereometric variable of mean cup depth was reduced by a mean value of 36±32 µm in the thin CCT group and by 4±36 µm in the thick CCT group, a difference that was statistically significant ($P=.003$, ANCOVA controlling for percentage IOP reduction) (**Table 2** and **Figure 1**). The maximum cup depth was reduced by 73±107 µm in the thin CCT group and by 4±89 µm in the thick CCT group, a difference that was statistically significant ($P=.02$, ANCOVA). The relationship between corneal thickness and shallowing of the cup was present in the OAG and OHT groups and was not significantly different between the groups ($P=.29$ and $P=.18$ for mean and maximum cup depths, respectively, ANCOVA) (**Table 3**).

Table 1. Characteristics of Patients With Open-angle Glaucoma and Ocular Hypertension*

Characteristic	Thin CCT Group (n = 16)	Thick CCT Group (n = 16)	*P* Value
CCT, µm	518 ± 32	587 ± 31	<.001
Age, y	64.0 ± 12.8	62.2 ± 12.4	.69
Cup-disc ratio			
Clinically determined	0.58 ± 0.24	0.56 ± 0.26	.84
Vertical (HRT)	0.46 ± 0.19	0.40 ± 0.21	.41
Area (HRT), mm²			
Disc	1.94 ± 0.38	2.11 ± 0.34	.20
Rim	1.02 ± 0.30	1.25 ± 0.41	.09
Cup depth (HRT), mm			
Mean	0.29 ± 0.12	0.28 ± 0.11	.64
Maximum	0.69 ± 0.27	0.64 ± 0.19	.53
Refractive error, diopters	−0.2 ± 2.1	0.1 ± 3.5	.82
Mean defect of visual field, dB†	−3.2 ± 4.1	−4.1 ± 6.9	.68
Maximum IOP before reduction, mm Hg‡	29 ± 4	29 ± 5	.82
IOP before reduction, mm Hg	24.6 ± 5.6	24.8 ± 4.8	.9
% IOP reduction§	38 ± 13	30 ± 12	.09

Abbreviations: CCT, central corneal thickness; HRT, Heidelberg retina tomography; IOP, intraocular pressure.
*Data are given as mean ± SD unless otherwise indicated.
†Mean mean defect on recent Humphrey automated perimetry.
‡Maximum recorded untreated IOP for the eye.
§At the time of the second imaging session.

Table 2. Change in Topography After Sustained Intraocular Pressure (IOP) Reduction Among Combined Patients With Open-angle Glaucoma and Ocular Hypertension*

Cup Depth, µm	Thin CCT Group (n = 16)	Thick CCT Group (n = 16)	Unit Difference	*P* Value†
Mean	−36 ± 32	−4 ± 36	−32	.003
Maximum	−73 ± 107	−4 ± 89	−69	.02

Abbreviation: CCT, central corneal thickness.
*Data are given as mean ± SD unless otherwise indicated.
†Analysis of covariance controlling for percentage IOP reduction.

We also looked for significant changes of cup depth in individual eyes. The standard deviation of cup depth for the 3 images performed at each of 2 sessions (before and after IOP reduction) was calculated. Then, the number of eyes in which the cup depth changed by more than 4 SDs for that eye was calculated. One eye for which we were unable to locate the original images was excluded from this analysis. For mean cup depth, 8 of 15 eyes showed significant shallowing in the thin CCT group, while 3 of 16 eyes showed significant shallowing in the thick CCT group, a difference that was significant by χ^2 analysis ($P=.04$). The same analysis for maximum cup depth yielded 4 of 15 eyes showing at least 4-SD shallowing in the thin CCT group compared with 1 of 16 eyes in the thick CCT group, a difference that was not significant ($P=.1$). We further confirmed the difference in topographical changes by performing the Mann-Whitney rank order test for changes of mean and maximum cup depth. This test confirmed that, compared with the thick CCT group, the thin CCT group had significantly greater reductions in mean cup depth ($P=.02$), while the greater reduction in maximum cup depth in the thin CCT group did not reach statistical significance ($P=.13$).

Smaller improvements in neuroretinal rim blood flow were seen in patients with OAG and OHT with thinner corneas compared with those with thicker corneas. This difference was statistically significant in the patients with OAG and OHT and remained significant after controlling for percentage IOP reduction ($P=.04$, ANCOVA). This difference was significant in the OAG group but not in the OHT group (**Table 4** and **Figure 2**). We also looked for significant changes of rim flow in individual eyes. The standard deviation of rim flow for the 5 images performed at each of 2 sessions was calculated. Then, the number of eyes in which the rim flow changed by more than 4 SDs for that eye was calculated. Seven of 16 eyes showed significant increases in rim flow in the thick CCT group, while 2 of 16 eyes showed such increases in the thin CCT group, a difference that was significant by χ^2 analysis at $P=.05$. Using a cutoff of 3 SDs gave a more significant χ^2 result of $P=.01$ (9 of 16 in the thick CCT group vs 2 of 16 in the thin CCT group).

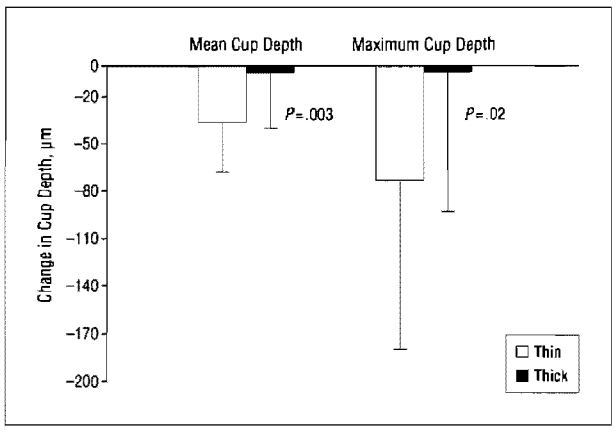

Figure 1. Change in topography.

COMMENT

The results of this preliminary study suggest that patients with OHT and OAG with thin central corneas have greater forward displacement of the base of the cup, a surrogate marker for lamina cribrosa position, following IOP reduction than their cohorts with thicker central corneas. Patients with thin central corneas also seem to have smaller improvements in neuroretinal rim blood flow after IOP reduction than patients with thicker central corneas.

A thin central cornea is emerging as a major risk factor for severity of OHT and OAG.[23-27] Diurnal and long-term IOP fluctuations are also a major risk factor for progression in OAG.[32-34] These results suggest that a thin central cornea may be a marker for physiological differences in the biomechanical properties of the lamina cribrosa. In other words, it may be that a thin central cornea is connected to a thin sclera, which, in turn, is connected to a thin lamina. Assuming identical material properties, a thin lamina should demonstrate greater compliance (less rigidity) than a thick lamina. A thin lamina should then manifest greater displacement in response to diurnal or therapeutic IOP fluctuations. Greater laminar displacement could lead to increased damage to adjacent axons by different mechanisms.[35]

Larger increases in rim blood flow after IOP reduction were observed in the thick CCT group. Because patients with thick central corneas may have a reduced risk of progressing or of reaching an advanced state of glaucoma,[23-27] this finding suggests that improved blood flow in response to therapy may be a good prognostic sign in glaucoma. In patients with thin central corneas, it is conceivable that the vasculature has become more damaged due to repetitive movements of the more compliant lamina. In these patients, the vasculature may be less able to respond to IOP reduction with a beneficial increase in blood flow. Smaller increases in optic nerve head blood

Table 3. Change in Topography After Sustained Intraocular Pressure Reduction Among Patients With Open-angle Glaucoma (OAG) vs Ocular Hypertension (OHT)*

	Change in Mean Cup Depth, µm			Change in Maximum Cup Depth, µm		
Diagnosis	Thin CCT Group	Thick CCT Group	Combined Groups	Thin CCT Group	Thick CCT Group	Combined Groups
OAG (n = 16)	−43 ± 29	−7 ± 41	−25 ± 39	−95 ± 104	−22 ± 88	−59 ± 101
OHT (n = 16)	−29 ± 34	−1 ± 33	−15 ± 35	−50 ± 110	14 ± 92	−18 ± 104
Combined (n = 32)	−36 ± 32	−4 ± 36	−20 ± 37	−73 ± 107	−4 ± 89	−38 ± 103

Abbreviation: CCT, central corneal thickness.
*Data are given as mean ± SD.

Table 4. Change in Neuroretinal Rim Blood Flow After Sustained Intraocular Pressure Reduction*

Diagnosis	Thin CCT Group, AU	Thick CCT Group, AU	P Value
OAG (n = 16)	46 ± 82	150 ± 106	.048
OHT (n = 16)	23 ± 83	71 ± 110	.34
Combined (n = 32)	35 ± 80	110 ± 111	.04

Abbreviations: AU, arbitrary units; CCT, central corneal thickness; OAG, open-angle glaucoma; OHT, ocular hypertension.
*Data are given as mean ± SD unless otherwise indicated.

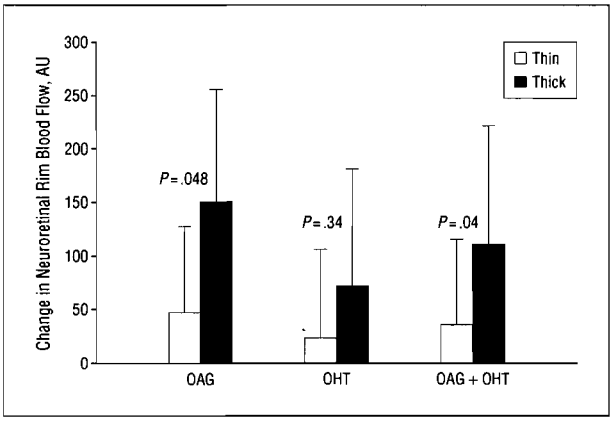

Figure 2. Change in neuroretinal rim blood flow. AU indicates arbitrary units; OAG, open-angle glaucoma; and OHT, ocular hypertension.

flow may also be present on IOP reduction because the microvasculature passing through the lamina cribrosa may become compressed by the large forward displacement of the laminar sheets. Laminar sheet compression is common in glaucoma.[16] These data suggest an interrelationship between the mechanical and vascular properties of the optic nerve head.

However, our data on neuroretinal rim blood flow may, in fact, be misleading. In the eyes with more compliant laminas, it is possible that laminar (as opposed to neuroretinal rim) blood flow after IOP reduction was greatly increased. This increase in laminar blood flow (not measured by our method) may have manifested as a less impressive increase in neuroretinal rim blood flow because of shunting. Future research should examine lamina cribrosa blood flow and neuroretinal rim blood flow.

A review of the literature suggests that, after an initial hypercompliant phase, the lamina cribrosa becomes more rigid in glaucoma.[1-9,14,15,35,36] One interpretation of these findings is that increased laminar rigidity contributes to axonal loss. Another interpretation is that increased laminar rigidity follows axonal loss. Findings from the present study suggest that patients with thick central corneas have a more rigid lamina cribrosa. Because other studies[23-27] have demonstrated a lower risk of progression in patients with thick central corneas, increased laminar rigidity may be a biological response that is protective to axons in this disease. Although the mechanisms of this protection remain unknown, the results of our study suggest that improved blood flow to the neuroretinal rim after IOP reduction may be involved in this protective effect.

Our findings also suggest a potential method for determining the risk level for an individual patient with glaucoma. Patients with high lamina cribrosa mobility or poor vascular response to IOP reduction may be at greater risk of progressive disease and may be targeted for more aggressive or alternate therapies. Although the results presented herein are preliminary and the mechanistic links are speculative, they serve as a conceptual framework for more detailed future studies.

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