Analysis of Angiographies in Human Healthy Eyes and in Open-angle Glaucoma

Retinal Mean Transit Time and Optic Nerve Head Circulation

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Abstract

Purpose of the studies was to develop a more robust technique to determine retinal mean transit time in healthy and in glaucoma eyes and to evaluate the circulation of the optic nerve head in glaucoma patients.

The retinal mean transit time impulse-response method was evaluated in human healthy eyes and normal values and reproducibility were tested.

Fluorescein and indocyanine green angiographies were recorded and the pictures were analyzed to obtain retinal mean transit time and to evaluate the proportion of low-fluorescent pixels of the optic nerve head in the glaucoma patients. Visual field defects were correlated to loss of neuroretinal rim area.

A disturbed circulation was observed in the glaucoma patients, whether primary or secondary to loss of nerve fibre tissue can not be determined from these studies.

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To my beloved mother and son
List of papers


Paper 3: Bjärnhall G, Tomic L, Sperber GO, Mäepea O and Alm A. The effect of elevated intraocular pressure on the retinal circulation in open-angle glaucoma and ocular hypertension. A study on retinal mean transit time. Manuscript

Paper 4: Bjärnhall G, Tomic L, Sperber GO, Mäepea O and Alm A. The optic nerve head in open-angle glaucoma and ocular hypertension. A study with fluorescein and ICG angiography. Manuscript
## Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>11</td>
</tr>
<tr>
<td>Anatomy of retinal and choroidal circulation</td>
<td>11</td>
</tr>
<tr>
<td>Vasculature of the anterior optic nerve and the peripapillary region</td>
<td>12</td>
</tr>
<tr>
<td>Ocular perfusion pressure</td>
<td>12</td>
</tr>
<tr>
<td>Autoregulation of retinal blood flow</td>
<td>13</td>
</tr>
<tr>
<td>Autoregulation in open-angle glaucoma</td>
<td>14</td>
</tr>
<tr>
<td>Pathogenesis of open-angle glaucoma</td>
<td>15</td>
</tr>
<tr>
<td>Open-angle glaucoma risk factors</td>
<td>15</td>
</tr>
<tr>
<td>Ocular circulation and open-angle glaucoma</td>
<td>16</td>
</tr>
<tr>
<td>Evaluation of the circulation of the optic nerve head in open-angle glaucoma</td>
<td>17</td>
</tr>
<tr>
<td>Evaluation of ocular circulation in open-angle glaucoma, a summary of techniques</td>
<td>18</td>
</tr>
<tr>
<td>Purposes</td>
<td>19</td>
</tr>
<tr>
<td>Study 1</td>
<td>19</td>
</tr>
<tr>
<td>Study 2</td>
<td>19</td>
</tr>
<tr>
<td>Study 3</td>
<td>19</td>
</tr>
<tr>
<td>Study 4</td>
<td>19</td>
</tr>
<tr>
<td>Methods</td>
<td>20</td>
</tr>
<tr>
<td>Fluorescein angiography and retinal mean transit time</td>
<td>21</td>
</tr>
<tr>
<td>Recording the fluorescein angiography of healthy volunteers or patients</td>
<td>24</td>
</tr>
<tr>
<td>Calculation of mean transit time</td>
<td>25</td>
</tr>
<tr>
<td>Summary of AVP, MTT, MTT\text{SLOPE} and MTT\text{IR}</td>
<td>28</td>
</tr>
<tr>
<td>Humphrey perimetry</td>
<td>29</td>
</tr>
<tr>
<td>Heidelberg retinal tomography</td>
<td>30</td>
</tr>
<tr>
<td>Goldmann applanation tonometry</td>
<td>30</td>
</tr>
<tr>
<td>Slit-lamp examination</td>
<td>30</td>
</tr>
<tr>
<td>Determination of the distribution of low-fluorescent pixels in fluorescein and ICG angiography</td>
<td>31</td>
</tr>
<tr>
<td>Measurement of systemic blood pressure</td>
<td>32</td>
</tr>
<tr>
<td>Participants</td>
<td>32</td>
</tr>
<tr>
<td>Study 1</td>
<td>32</td>
</tr>
<tr>
<td>Study 2</td>
<td>32</td>
</tr>
<tr>
<td>Study 3 and 4</td>
<td>34</td>
</tr>
</tbody>
</table>
Results...........................................................................................................36

Study 1: Analysis of retinal mean transit time from fluorescein
angiography in human eyes: normal values and reproducibility............36

Study 2: Retinal mean transit time in patients with primary open-angle
 glaucoma and normal tension glaucoma..................................................37

Study 3: The effect of elevated intraocular pressure on the retinal
circulation in open-angle glaucoma and ocular hypertension. A study on
retinal mean transit time...........................................................................37

Study 4 The optic nerve head in open-angle glaucoma and ocular
hypertension. A study with fluorescein anf ICG angiography .................38

Discussion.....................................................................................................40

Conclusions...................................................................................................42

Acknowledgments.........................................................................................43

Sammanfattning på svenska..........................................................................44
Abbreviations

AVP  Arteriovenous passage time
BF   Blood flow
BP   Blood pressure
FAI  Fluorescein angiography imaging
GHT  Glaucoma hemifield test
HRA  Heidelberg retinal angiograph
HRT  Heidelberg retinal tomograph
ICG  Indocyanine green
IOP  Intraocular pressure
MAP  Mean arterial blood pressure
MD   Mean deviation
MTT  Mean transit time
NTG  Normal tension glaucoma
OPP  Ocular perfusion pressure
Pa   Pressure in the arteries
Pv   Pressure in the veins
PEX  Psedoexfoliations
POAG Primary open-angle glaucoma
R    Vascular resistance
SLO  Scanning laser ophthalmoscope
Ocular blood flow has been assessed by various techniques, each technique investigating a defined parameter of retinal or choroidal circulation. Several methods to investigate ocular hemodynamics are invasive and not suitable in human eyes. Other methods measure different aspects of retinal or choroidal blood flow indirectly.

In the present studies a new technique to determine retinal blood flow transit times has been used to study the retinal circulation in normal eyes and in eyes with open angle glaucoma.

A new unbiased method was used to evaluate low fluorescent pixels from digitised frames from angiographies of the optic nerve head and relate them to visual field defects.

Anatomy of retinal and choroidal circulation

The retinal and choroidal vessels are two separate systems nourishing the structures of the eye.\textsuperscript{1}

The ophthalmic artery is the first branch of the internal carotid artery and the only extracranial branch. The major branches of the ophthalmic artery are to the extraocular muscles, the central retinal artery, the posterior ciliary arteries and the anterior ciliary arteries.

The central retinal artery penetrates the optic nerve approximately 10 mm behind the globe and emerges on the optic disc where it usually divides into four branches.

The central retinal artery supplies the inner two-thirds of the retina. It also supplies the superficial nerve fiber layer of the optic disc and takes part in supplying the optic nerve behind the lamina cribrosa.

The posterior ciliary arteries supply the outer layers of the retina (photoreceptors, horizontal and bipolar cells), the ciliary body and the iris. The outer layer of the retina contains no blood vessels.

The posterior ciliary arteries each divide into one long posterior ciliary artery and seven to ten short posterior ciliary arteries. The latter vessels then penetrate the sclera close to the optic nerve.

The short posterior ciliary arteries form the choroid that also leaves small branches to the laminar part of the optic nerve. Sometimes an arterial circle, the arterial circle of Zinn and Haller, can be identified within the perineural
sclera. It is formed by the confluence of branches of the short posterior ciliary arteries.

The anterior ciliary arteries and the long posterior ciliary arteries supply the anterior choroid, the iris and the ciliary body.

Vasculature of the anterior optic nerve and the peripapillary region

The blood circulation of the optic nerve emerges from different systems, the arteries of the pia mater, the ciliary circulation, the choroidal and the retinal blood vessels.

The anterior optic nerve head can be divided into four layers, the superficial nerve fiber region, the prelaminar region, the lamina cribrosa and the retrolaminar region.

Branches from the central retinal artery supply the superficial nerve fiber layer and the optic nerve head.

The prelaminar region and the lamina cribrosa are nourished by branches of the short posterior ciliary arteries, the circle of Zinn and Haller and branches from the choroidal arteries.

The peripapillary choriocapillaris does not supply the prelaminar region.

The retrolaminar region is supplied the arterioles from the central retinal artery and from the pia mater.

The central retinal vein drains the anterior optic nerve.

Ocular perfusion pressure

The perfusion pressure of the eye is equal to the difference between the pressure in the arteries (Pa) entering the eye and the pressure in the veins (Pv) leaving the eye. The following equation describes the relationship between ocular blood flow (BF), ocular perfusion pressure (OPP = PA - PV) and the resistance in the vascular bed (R), Figure 1.

\[
BF = \frac{Pa - Pv}{R}
\]

Figure 1. Blood flow (BF), Pa (pressure of the artery), Pv (pressure of the vein) and R (vascular resistance)
The pressure within the retrobulbar arteries cannot be measured in humans. The mean arterial blood pressure (MAP) is used as an estimation of the pressure in the ocular arteries, Figure 2.

\[
\text{MAP} = \text{DBP} + \frac{1}{3} (\text{SBP} - \text{DBP})
\]

Figure 2. Mean arterial pressure (MAP), Diastolic blood pressure (DBP) and Systolic blood pressure (SBP)

In animal studies, nonhuman primates, the difference between MAP and the mean pressure in the anterior ciliary arteries entering the ocular tissue is 20-25 mmHg. The pressure in the arteries entering the human eye is estimated to 60-70 mmHg. The intraocular pressure (IOP) can be used as a measurement of the pressure in the veins (Pv) leaving the eye since the IOP and the Pv is almost equal at IOP at normal and elevated (IOP>21 mmHg) levels. The normal perfusion pressure of the eye is approximately 50mmHg.

OPP (Pa-Pv) can be reduced in two ways, by reducing the blood pressure or by elevation of the IOP. In glaucoma with elevated IOP the OPP is reduced in cases where the autoregulation of the retinal blood flow is impaired. The ocular blood flow is reduced when there is a decrease in OPP if the vascular resistance (R) remains unchanged.

**Autoregulation of retinal blood flow**

Autoregulation is defined as the maintenance of stable BF when there is a change in the OPP. Autoregulation of the retina has been demonstrated in animal studies. The regulation of vascular resistance and thereby the autoregulation are probably depending on two pathways, the myogenic and the metabolic, involving factors released from the retinal metabolism and the vascular endothelium. The vascular tone of the arterioles and the capillaries adapts to changes in the OPP or the metabolic needs of the retinal tissue. In the human eye BF through the retina and the optic nerve is autoregulated, Figure 3, when the IOP is increased. Animal experiments, in cats and monkeys, have shown reduction in choroidal blood flow in response to moderate IOP elevations. The retina lacks sympathetic innervation and the blood retinal barrier prevents non-lipid soluble substances in blood to reach the vascular smooth muscles. No or minimal autoregulation is found in the choroidal circulation, the BF is more dependent of the OPP.
Autoregulation of the blood flow of the prelaminar area of the optic nerve has been found in normal human and in monkey eyes.\textsuperscript{14, 15} The optic nerve head is supplied by branches from choroidal and ciliary arteries, the vascular endothelium of these vessels are probably involved in mediating the vascular resistance.\textsuperscript{16, 18}

**Figure 3.** Autoregulation of retinal blood flow.

**Autoregulation in open-angle glaucoma**

The retinal circulation autoregulates to approximately IOP 27-30 mmHg\textsuperscript{11, 12, 17} in normal human eyes. A suction cup was used to increase the IOP in several studies. Laser Doppler velocimetry was used\textsuperscript{11} by Riva et al in normal eyes and IOP was increased. In glaucomatous eyes the IOP level where the autoregulation is keeping the retinal BF constant is lower\textsuperscript{19}, approximately IOP 25 mmHg. In this study by Grunwald et al the IOP was elevated with a scleral suction cup and blue field simulation technique was used to study the macular blood flow. Thus retinal BF may be more affected by moderate changes in IOP in eyes primary open-angle glaucoma (POAG).
Pathogenesis of open-angle glaucoma

POAG is an optic neuropathy with a characteristic pattern of damage to the optic nerve and a concomitant loss of the visual field. The etiology of the optic nerve damage is still debated.

There are two different theories about the pathogenesis of optic nerve damage in glaucoma; the mechanical and the vascular theory. The mechanical theory postulates an elevated intraocular pressure as an important factor. The optic nerve fibers are damaged through the elevated IOP at the level of the lamina cribrosa where the axons are misaligned against the edge of the sclera. Animal studies also showed optic nerve head damage after elevation of IOP. It was also found in the Barbados Eye Study that higher IOP contributes to the risk of developing open-angle glaucoma in human eyes. Still IOP is not elevated in all eyes with OAG, but recent studies have demonstrated that reducing IOP slows down progression also in eyes with OAG and untreated normal IOP.

Animal studies have shown death of retinal ganglion cells due to apoptosis, the starting mechanism of apoptosis is unknown, but ischemia could contribute.

The vascular theory postulates a disturbance in the ocular blood flow. According to this theory dysregulation or a reduction of the blood supply to the optic nerve could contribute to the glaucomatous damage.

Open-angle glaucoma risk factors

In the Barbados Eye Study the incidence of POAG in nine years was 4.4% (0.5% per year) in a population of African descent. In studies of European populations incidence rates 0.1% to 0.3% per year have been found. The wide spread in incidence rate may be due to differences in the prevalence of pseudoexfoliations (PEX) which is an independent risk factor for the development of OAG.

Several risk factors have been identified; demographic, ocular as well as systemic. Thus older age, African descent and family history increase the risk of OAG. Ocular risk factors include elevated IOP, PEX of the lens capsule, myopia and thinner corneas. Systemic risk factors have been less well documented, but large clinical trials indicate that heart disease, low systolic blood pressure and low ocular perfusion pressure all increase the risk of developing open-angle glaucoma. There are several arguments supporting the effect of elevated IOP on the optic nerve, including the markedly increased risk of chronic open-angle glaucoma in otherwise normal eyes with elevated IOP, the development of secondary glaucoma in eyes with secondary elevation of the IOP, the effect of increased IOP on the optic nerve in several animal models of glaucoma, and the demonstration...
that elevated IOP affects axoplasmic flow in the optic nerve of monkeys.\textsuperscript{37, 38} Still, in population surveys more than one third of all eyes with OAG have an IOP within normal limits, normal tension glaucoma (NTG). Thus a purely mechanical theory based on an elevated IOP is not able to satisfactorily explain all cases of OAG. A vascular theory has been proposed as an alternative.\textsuperscript{39, 40} This theory is supported by recent clinical trials demonstrating systemic circulatory risk factors for the development of open-angle glaucoma.\textsuperscript{32, 36}

Ocular circulation and open-angle glaucoma

Clinical trials have reported several systemic circulatory risk factors for the development of OAG.\textsuperscript{32, 36} Quantitative measurements of ocular blood flow in the human eye are not yet possible, but several non-invasive techniques to study various aspects of ocular circulation have been developed and are at present used in clinical research. These techniques usually measure velocity of red or white blood cells or a tracer (fluorescein) and cannot be translated into blood flow unless the vascular volume is known. Recent technical improvements provide vessel diameter and velocity in single retinal vessels\textsuperscript{41, 42}, but determination of total retinal blood flow will still be impractical in humans.

Despite the limitations of the clinical techniques they all provide some information on a particular vascular bed and all of them have been used to study ocular blood flow in glaucoma. Studies on retinal circulation in eyes with glaucoma have shown lower flow velocities with blue field simulation\textsuperscript{43} Color Doppler examinations have demonstrated reduced velocity in the retrobulbar vessels.\textsuperscript{44, 45, 46, 47, 48} Reduced pulsatile ocular blood flow has been reported.\textsuperscript{49, 50, 51} Also increased arteriovenous passage time (AVP), determined from fluorescein angiograms, was found in several studies.\textsuperscript{48, 52, 53}

Disturbed circulation of the optic nerve head and peripapillary retina determined with scanning laser flowmetry.\textsuperscript{54, 55, 56, 57} A possible correlation between the degree of disturbed ocular or retrobulbar circulation and the level of glaucomatous damage, as estimated by the mean deviation of the visual field, has been evident in several studies.\textsuperscript{58, 59, 60} Such an alternative mechanism for damaging the optic nerve is particularly interesting in eyes with IOP within normal limits, normal-tension glaucoma (NTG). Fluorescein angiography\textsuperscript{61}, color Doppler imaging\textsuperscript{46, 47, 58, 62, 63} and scanning laser flowmetry\textsuperscript{64} have all been used to examine whether there is a difference between eyes with POAG and eyes with NTG. No differences or small differences were observed and the results have not been conclusive. Thus, whether disturbed ocular blood flow is more pronounced in NTG than in POAG is still an open question.
Fluorescein angiography is still the major clinical technique for studies of the retinal circulation. Spaeth\textsuperscript{65} performed a prospective fluorescein angiography study in POAG, NTG, glaucoma suspects and healthy controls. Localised hypoperfusion of the optic nerve head was observed in the POAG and NTG groups but rarely in the healthy group, a statistically significant difference was found. There was no significant difference between the glaucoma suspects and the healthy controls. The area of hypoperfusion was usually inferior temporally. Persisting hypoperfusion of the optic nerve head in the glaucoma patients was significantly correlated with visual field loss.

IOP reduction in about one-third of the glaucoma patients improved disc perfusion. Spaeth also observed prolonged optic disc filling time in glaucoma patients compared to the controls.

**Evaluation of the circulation of the optic nerve head in open-angle glaucoma**

The first study describing reduced fluorescence of the optic nerve head in POAG was published by Hayreh and Walker in 1967.\textsuperscript{95} Hypofluorescence of the optic nerve head in patients with normal IOP was observed. Several investigators have later confirmed this observation.\textsuperscript{65, 96, 97, 98, 99,100, 101} Absolute fluorescein angiographic filling defects of the optic nerve head in POAG and NTG compared to normal subjects have been described.\textsuperscript{102, 103} A few studies reports filling defect of the optic nerve head with ICG.\textsuperscript{104, 105}

Peripapillary atrophy has been described by Jonas et al\textsuperscript{106} in glaucoma patients, a peripheral \( \alpha \) zone with irregular pigmentation and a central \( \beta \) zone with visible sclera and visible large choroidal vessels. Progression of glaucomatous visual field defects was correlated to larger \( \beta \) zone of parapapillary atrophy and smaller neuroretinal rim area.\textsuperscript{106} Peripapillary atrophy has also been reported in an elderly Chinese population without glaucoma.\textsuperscript{110} The \( \alpha \) zone and the \( \beta \) zone were widest in the peripillary temporal region, and the size of the zones was correlated to optic disc size, myopia and age.
Evaluation of ocular circulation in open-angle glaucoma, a summary of techniques

There are several indirect, noninvasive techniques evaluating different aspects of ocular circulation, Table 1. The techniques have limitations and no single technique can give a total picture of the ocular circulation.

**Techniques and what they measure**
- Fluorescein angiography: plasma velocity in main retinal vessels
- Retinal vessel diameter: vessel diameter from fluorescein angiography
- Indocyanine green angiography (ICG): choroidal filling time
- Laser Doppler velocimetry: erythrocyte velocity in retinal veins
- Laser Doppler flowmetry: velocity in optic nerve head capillaries
- Pulsatile ocular blood flow: fundus pulsation
- Color Doppler imaging: blood velocity in extraocular vessels
- Blue field simulation: leukocyte velocity in macular capillaries

**Table 1. Methods of evaluating ocular circulation in open–angle glaucoma**

<table>
<thead>
<tr>
<th>Author</th>
<th>Method</th>
<th>POAG/NTG</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jonas et al⁸²   1989</td>
<td>Retinal vessel diameter</td>
<td>POAG</td>
<td>Parapapillary retinal vessel diameter decreased</td>
</tr>
<tr>
<td>Wolf et al⁸³    1993</td>
<td>Fluorescein angiography</td>
<td>POAG</td>
<td>AVP increased</td>
</tr>
<tr>
<td></td>
<td>1. AVP</td>
<td></td>
<td>Mean dye velocity decreased</td>
</tr>
<tr>
<td></td>
<td>2. Mean dye velocity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chung et al⁸⁴   1998</td>
<td>ICG angiography</td>
<td>NTG</td>
<td>Heterogenous filling</td>
</tr>
<tr>
<td></td>
<td>1. Early choroidal filling</td>
<td></td>
<td>(Homogenous filling in normal eyes)</td>
</tr>
<tr>
<td></td>
<td>2. Peripapillary choroidal</td>
<td></td>
<td>Delayed filling</td>
</tr>
<tr>
<td>Chung et al⁸⁵   1999</td>
<td>Doppler flowmetry</td>
<td>NTG</td>
<td>Blood flow decreased</td>
</tr>
<tr>
<td>Fontana et al⁸⁶ 1998</td>
<td>Pulsatile ocular blood flow</td>
<td>NTG</td>
<td>Decreased</td>
</tr>
<tr>
<td>Galassi et al⁸⁷ 1992</td>
<td>Color Doppler</td>
<td>POAG</td>
<td>Peak systolic velocities decreased in A. Ophthalmica</td>
</tr>
<tr>
<td>Sergott et al⁸⁸ 1994</td>
<td>Color Doppler</td>
<td>POAG</td>
<td>Peak systolic velocities and end diastolic velocities decreased in A. Ophthalmica and Aa. ciliares posterior</td>
</tr>
<tr>
<td>Harris et al⁸⁹  1994</td>
<td>Colour Doppler</td>
<td>NTG</td>
<td>A. Ophthalmica: increased</td>
</tr>
<tr>
<td></td>
<td>1. Resistant indices</td>
<td></td>
<td>A. Ophthalmica: decreased</td>
</tr>
<tr>
<td></td>
<td>2. End diastolic velocities</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Königsreuther and Michelson⁹⁰ 1994</td>
<td>Color Doppler</td>
<td>POAG</td>
<td>Velocities decreased</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>A. centralis retinae</td>
</tr>
<tr>
<td>Grunwald et al⁹¹ 1984</td>
<td>Blue field simulation</td>
<td>POAG</td>
<td>Leukocyte velocity decreased</td>
</tr>
</tbody>
</table>

18
Purposes

Study 1
- To determine normal values and reproducibility of three different methods to quantify retinal transit times from fluorescein angiographies.
- To evaluate the correlations between three different methods to quantify retinal mean transit times

Study 2
- To examine if retinal MTT is different in eyes with NTG compared to POAG.
- To determine if the degree of optic nerve damage has an effect on retinal MTT.

Study 3
- To determine if retinal MTT differs between the two eyes in patients with newly detected OAG or ocular hypertension (OH) in one eye.
- To determine if retinal MTT is affected when IOP is normalized/reduced in eyes with previously untreated OAG or OH.

Study 4
- To determine the distribution of low-fluorescent pixels from digitized frames of fluorescein and ICG angiographies in optic discs with various degrees of glaucomatous damage.
- To compare structural and functional damage by comparing optic disc structure and visual fields.
- To study peripapillary atrophy
Methods

Methods in study number one:
- Fluorescein angiography, retinal MTT and AVP
- Slit-lamp examination of the anterior and posterior segment

Methods in study number two:
- Fluorescein angiography, retinal MTT and AVP
- Humphrey perimetry
- Goldman applanation tonometry
- Slit-lamp examination of the anterior and posterior segment

Methods in study number three and four:
- Fluorescein angiography, retinal MTT and AVP study 3
- Fluorescein angiography, distribution of low-fluorescent pixels in the optic nerve head, study 4
- ICG angiography evaluation, distribution of low-fluorescent pixels in the optic nerve head, study 4
- Evaluation of peripapillary atrophy from red free pictures and Heidelberg retinal tomography (HRT) images, study 4
- Humphrey perimetry
- Heidelberg retinal tomography
- Goldman applanation tonometry
- Slit-lamp examination of the anterior and posterior segment
- Measurement of systemic blood pressure, study 3
Fluorescein angiography and retinal mean transit time

A number of ophthalmological and systemic diseases affect the retinal circulation, either primarily or secondarily. Clinical evaluation of retinal circulation is mainly based on qualitative interpretation of fluorescein angiograms, but several attempts have been made to quantify retinal angiograms. One of these is to determine the mean transit time (MTT) of the retinal circulation. MTT is defined as the volume / flow ratio of a vascular bed.\textsuperscript{69, 70} It corresponds to the average time spent by a tracer in the vascular bed or the time it takes to renew the blood within the vascular bed. MTT is expressed in seconds.

Hickam and Frayser\textsuperscript{71} in 1965 were the first to calculate MTT of the retinal circulation from dye curves constructed by densitometric analysis of a sequence of frames from fluorescein angiograms. They reported a MTT of 4.7 seconds in normal eyes.

Riva et al\textsuperscript{72} in 1978 collected the fluorescent light with a circular aperture slightly larger than the vessel. Conventionally MTT is calculated from curves obtained by semilogarithmic extrapolation of the down-slope of the dye curves.\textsuperscript{69, 70} Riva and co-workers could show that the grey scale level of the intravascular fluorescein is a good estimate of the relative concentration of fluorescein in the vessels, as long as very high concentrations of fluorescein are not injected.\textsuperscript{72} Thus the arterial and venous curves represent the fluorescein concentration-time curves of the vessels, Figure 4.

Conventional techniques to calculate MTT from the down-slope of the dye curves by extrapolation from a semi-logarithmic plot, requires well defined dye curves and can in our experience not be done in a substantial number of clinical fluorescein angiographies.
AVP is the time between the appearance of fluorescein in the artery and the corresponding vein, Figure 5. Retinal circulation in retinal arterioles and venules is quantified by measurement of AVP, Wolf et al.\textsuperscript{73} The disadvantage of this measurement is that it estimates only the transit from artery to vein close to the optic disc, without taking into account transit in the peripheral part of the retina. It also introduces a small error since the fluorescence appears first in the center of the artery and can leave the artery first when the fluorescein has filled the artery completely and reached the peripheral parts of the vessel. This time delay depends on the shape of the bolus and is probably rather small.
The introduction of the scanning laser ophthalmoscope (SLO) permits analysis of several frames per second from an angiogram.

The light source of the SLO camera is a low power scanning argon laser beam, the beam illuminates the retina point by point. The result is a reduced retinal illumination and improved contrast of the pictures. Opacities of the lens and cornea are penetrated more easily by the argon beam. The SLO is a confocal instrument, thereby producing pictures with improved spatial resolution and contrast.
Recording the fluorescein angiography of healthy volunteers or patients

The procedure started with a rapid injection of 0.3-0.5 ml of a 25% solution of sodium fluorescein into the antecubital vein. This was followed by a 5 ml flush of saline solution (0.9 mg/ml) to obtain a well-defined fluorescein bolus.

The fluorescein angiographies were recorded with the Rodenstock Scanning Laser Ophthalmoscope (Rodenstock GmbH, München, Germany) with 20° field and fixed video and laser gains (study 1 and 2). They were recorded on a Panasonic 7355 SVHS videorecorder. The angiograms were digitized and a total of 128 pictures at a rate of 5 frames per second were sampled using a computerized image-analysing system developed by Sperber G. The Rodenstock SLO is no longer in use and for study 3 and 4 we have used the Heidelberg Retinal Angiograph 1 (Heidelberg Engineering GmbH, Heidelberg, Germany). This is a digital Scanning Laser Ophthalmoscope allowing 128 pictures at a rate of 6 pictures per second to be included in the analysis.

The recording started after the saline flush and ended 60 seconds later. The optic disc was centered during the recording. For the analysis of MTT and AVP the operator selected the main temporal arteries and their corresponding veins.

Circular regions of interest (ROI) were placed over the vessels, Figure 6. The ROI was placed over an artery and the corresponding vein close to the optic disc, two pairs of vessels were chosen, in the upper and lower temporal part of the retina. The mean value from these two pairs represents the MTT.
The first frame was taken one second before the first appearance of fluorescein in the central retinal artery. The brightness of the five pre-dye frames is at the same baseline level.

The grey levels from each pixel within a ROI were added and this was accepted as a measure of fluorescein concentration at that time point and the dye curves were plotted.

Calculation of mean transit time

Conventional calculation of MTT requires the reconstruction of the complete dye curves, from the first appearance of the dye in the vessel until all dye has disappeared. MTT corresponds to the difference in time between the centers of gravity of the arterial and venous dye curves, which makes the correct shape and length of the down-slope extremely important. Early recirculation of the dye distorts the downward slope so that this part of the curve has to be reconstructed by extrapolation from a semi-logarithmic plot. The short part of the down-slope that can be used sometimes makes this technique very inaccurate. The method developed by Stow and Hetzel\textsuperscript{76} in 1954, where the whole curve up to the recirculation is used, mean transit time slope (MTT\textsubscript{SLOPE}), to find the log-normal distribution function that optimally fits
the first passage is an improvement of pure extrapolation and has been used a number of times.\textsuperscript{72, 77}

An alternative technique for determining MTT from fluorescein angiographies based on an impulse-response analysis of frames from a scanning laser ophthalmoscope was developed by Sperber and Alm\textsuperscript{78}, mean transit time impulse-response (MTT\textsubscript{IR}). The advantage of this technique is that it is much less dependent on the shape of the dye curves. Analysis of MTT from the dye curves with the conventional semi-logarithmic extrapolation is sometimes impossible in healthy individuals as well as in patients with vascular pathology\textsuperscript{79} due to distortion of the dye curves, Figure 7.

The injection technique also has an important influence of the shape of the bolus.\textsuperscript{80, 81}

The MTT\textsubscript{IR} analysis is based on the general principle that the arterial concentration in each frame is regarded as a square-wave impulse and the resulting venous curve as the sum of identically shaped responses to this series of arterial square-wave impulses. An iterative procedure is used to find the impulse-response that yields the simulated venous curve closest to the recorded venous curve. Definition of MTT\textsubscript{IR} is given by the time co-ordinate of its centre of gravity, Figure 8.
Figure 7. Distorted dye curve

Figure 8. Mean transit time impulse-response
Summary of AVP, MTT, MTT_{SLOPE} and MTT_{IR}

**AVP**
AVP is the difference in time between the first appearance of fluorescein in the artery and the adjacent vein.
- Independent of recirculation
- Objective if the time when the dye concentration reached 10% of its maximum was used, AVP_{10%}
- Crude estimate of retinal MTT
- Demonstrates the shortest way through the vascular segment without any estimation of the peripheral circulation

**MTT**
MTT is a well-defined physiological parameter, volume/flow, it corresponds to the average time spent by a tracer in the vascular bed. MTT is expressed in seconds. MTT is not a measurement of retinal blood flow.
- A measurement of the retinal mean transit time including the peripheral parts of the retina

**Conventional MTT_{SLOPE}**
MTT_{SLOPE} is the difference in time coordinates of the centers of gravity of the extrapolated arterial and venous curves.
- Dependent on the shape of the curve
- Subjective, semi-logarithmic extrapolation of the down-slope of the dye curves
- Difficult to analyze curves with bad shape
- Tendency to overestimate the transit time
- Limited clinical usefulness

**MTT_{IR}**
An impulse-response analysis of the entire curve
- Independent of the shape of the curve
- Objective, no semi-logarithmic extrapolation of the down-slope of the dye curves
- Useful in clinical studies
- Inter- and intraindividual variability is smaller for MTT_{IR} compared to MTT_{SLOPE}
Humphrey perimetry

Standard automated static perimetry is the standard method of detecting and monitoring visual field defects in open-angle glaucoma. The minimum amount of light stimulus needed for the patient to detect a difference to the background is measured. The threshold sensitivity at different locations in the visual field is tested. Automated static perimetry gives information of visual sensitivity defined as: sensitivity=1/ threshold. Sensitivity is expressed in decibel, dB, the dB scale is a relative scale based on logarithmic intervals. Zero dB is defined as the most intense stimulus, the other values use the zero point as the reference.

Mean deviation (MD) gives information about the depression or elevation of the visual field, this value is based on the deviation from age matched normal values at each location in the visual field. The average of deviation values of sensitivity in the tested points of the visual field gives a measurement of functional loss.

The glaucoma hemifield test (GHT) detects localized visual field loss and visual field loss symmetric around the horizontal meridian. The upper and the lower hemispheres of the visual field are compared to another. There are five possible results that are printed:

1. GHT outside normal limits: The upper and lower hemispheres are quite different (found in less than 1% in normal eyes), therefore indicative of glaucoma damage.
2. GHT borderline
3. General reduction of sensitivity
4. Abnormally high sensitivity: indicates unreliable visual fields
5. Within normal limits

GHT identifies glaucomatous visual fields with high sensitivity and specificity.

Visual field loss due to glaucoma was identified using the criteria developed for detecting glaucomatous visual field loss for standard automated perimetry and short wavelength perimetry.

A pattern standard deviation worse than the normal 1% level
A GHT outside normal limits
One hemifield cluster worse than the normal 1 % level
Two hemifield clusters worse than the normal 1% level
Four abnormal (P< 0.05%) locations
Five abnormal locations (P <0.05 %) on the pattern deviation probability plot

In study 2 visual fields of the study eye were examined at one occasion with the Humphrey field analyzer (30-2 threshold program, SITA strategy; Carl Zeiss Meditec, Dublin, CA, USA) unless an equivalent examination had been carried out within the previous three months.
In study 3 and 4 (the same group of patients) visual fields of both eyes were examined before IOP reduction with the Humphrey field analyzer (central 24-2 threshold program, SITA strategy, Carl Zeiss Meditec, Dublin, CA, USA).

Heidelberg retinal tomography

The Heidelberg retinal tomography 3 (HRT; Heidelberg Engineering GmbH, Heidelberg, Germany) is a confocal scanning laser that provides 3-dimensional pictures of the optic nerve head. Quantitative assessment of the optic nerve head topography globally and in predefined segments and quantification of structural changes was obtained. A description of the present morphological state of the optic nerve head or detection of progression of optic nerve fibre loss is the goal of the examination.

The patients in study 3 and 4 (same group of patients) were examined at the first study visit before IOP reduction, both eyes were examined in 19 patients (one patient withdrew from the study after the first visit).

Three HRT images were acquired and only images with a high quality score as defined by HRT 3 were accepted.

Neuroretinal rim area (mm$^2$) for the whole disc and for the superior and inferior sectors of the disc was used in study 3 and 4.

Goldmann applanation tonometry

The IOP was measured in study 2 at the study visit and in study 3 and 4 (same group of patients) before and after topical IOP reduction with latanoprost in the study eye. Topical Lidocaine-fluorescein (40mg/ml+2,5 mg /ml), one drop was administered before measurement.

The Goldmann applanation tonometer was fixed on a slit-lamp (Haag-Streit).

Slit-lamp examination

The participants underwent slit-lamp (Haag-Streit) examination of the anterior and posterior segment.
Determination of the distribution of low-fluorescent pixels in fluorescein and ICG angiography

The fluorescein angiography was recorded as described previously. The ICG angiography was performed of the study eye before IOP reduction in seventeen eyes, one patient withdrew from the study, and two patients were sensitive to seafood. ICG 12.5 mg was diluted in 2 ml of sterile water and injected intravenously followed by a flush of 0.9% saline solution. The angiograms were recorded with the HRA1 camera.

The optic nerve head border was identified and outlined by the operator on the picture with the brightest fluorescence. The total number of pixels of this area was calculated using a computerized image-analysing system developed by one of the authors (G.O. Sperber). The program records the fluorescence (grey scale) of all pixels and discards all pixels except the 10% with the lowest intensity of fluorescence, Figure 8. The program also calculates how large a proportion, in per cent, of these low-fluorescent pixels that are located in the upper and lower half of the optic disc respectively. The distribution of low-fluorescent pixels in the optic nerve head was examined in study 4 in 20 patients before IOP reduction and in 19 patients after IOP reduction with topical latanoprost in the study eye.

Figure 8. Fluorescein angiography of optic nerve head showing the distribution of low-fluorescent pixels within the optic disc.
Measurement of systemic blood pressure

The blood pressure was measured with the patient in a sitting position after 10 minutes rest on the right arm. Diastolic and systolic blood pressure was registered with an automatic device (Omron, HEM-705C; Matsusaka, Matsusaka City, Japan).

The blood pressure was measured in study 3 before and after IOP reduction.

Participants

Study 1, 2, 3 and 4: the local Ethics Committees approved the projects and study protocols and the participants were informed according to the Helsinki Declaration.

Study 1

Twenty-nine healthy volunteers, 16 men and 13 men participated, mean age was 29.4 years (range 21-44 years). The participants had neither systemic nor ocular diseases and these persons used no medications.

There was no allergy in the group of participants.

Study 2

Patients followed for POAG (14 patients) or NTG (26 patients) at the Department of Ophthalmology, University Hospital, Hiroshima, Japan participated (Table 2 and 3). The diagnosis of POAG was based on glaucomatous change of the optic disc and/or glaucomatous visual field defect in an eye where IOP was > 21 mmHg without treatment. NTG was defined as an eye with glaucoma where IOP was ≤ 21 mmHg without treatment.

Exclusion criteria

- Active retinal disease
- Uveitis
- Intraocular surgery within three months
- Diabetes mellitus
- Pregnancy
- Breastfeeding
- Allergy

Systemic disease in the patients (n=3)

- Cushings disease one patient requiring systemic medication
- Essential hypertension one patient requiring systemic medication
• Essential hypertension and bipolar disease one patient requiring systemic medication

No systemic disease, no systemic medications (n=37)
• 37 patients

Concomitant ocular disease (n=4)
• Dry eye one patient
• Superficial punctate keratitis two patients
• Cataract one patient

Table 2. Baseline characteristics of patients at fluorescein angiography

<table>
<thead>
<tr>
<th></th>
<th>POAG (n=14)</th>
<th>NTG (n=26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>14</td>
<td>26</td>
</tr>
<tr>
<td>Male:Female</td>
<td>5:9</td>
<td>9:17</td>
</tr>
<tr>
<td>Age years</td>
<td>Mean 56.6 (range 30-76)</td>
<td>Mean 56.3 (range 43-74)</td>
</tr>
<tr>
<td>IOP mmHg</td>
<td>Mean 15.4 (range 8-22)</td>
<td>Mean 15.5 (range 10-20)</td>
</tr>
<tr>
<td>Visual acuity</td>
<td>Mean 0.9 (range 0.01-1.5)</td>
<td>Mean 1.0 (range 0.5-1.5)</td>
</tr>
<tr>
<td></td>
<td>Mean -11.5</td>
<td>Mean -6.7</td>
</tr>
<tr>
<td>Mean deviation (dB)</td>
<td>(range -30.8- -1.4)</td>
<td>(range -20.5- -7)</td>
</tr>
</tbody>
</table>

Table 3. Glaucoma treatment at fluorescein angiography

<table>
<thead>
<tr>
<th>Treatment</th>
<th>POAG (n=14)</th>
<th>NTG (n=26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No glaucoma treatment</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>Adrenergic betareceptor antagonists</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Prostaglandin analogue</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Nipradilol</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Combination: adrenergic betareceptor antagonist/prostaglandin analogue</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Combination: prostaglandin analogue/dorzolamide/dipivalylepinephrine/nipradilol</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>
Study 3 and 4

Twenty patients with newly diagnosed open-angle glaucoma or ocular hypertension in one eye agreed to participate, 13 men and 7 women between 50 and 75 years of age. Table 4 presents the demographic data. Table 5 presents diagnosed systemic diseases and systemic medications. The studies took place at the Institution of Neuroscience/Ophthalmology, University Hospital, Uppsala, Sweden.

Exclusion criteria
- Active retinal disease
- Uveitis
- Intraocular surgery within three months
- Diabetes mellitus
- Pregnancy
- Breastfeeding
- Allergy

Concomitant ocular disease n=2
- Dry pigmentations macula no leakage on fluorescein angiography, no pathology in ocular coherence tomography both eyes n=1
- Strabismic amblyopia control eye n=1

Table 4. Demographic data participants study 3 and 4

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>POAG</th>
<th>PEX</th>
<th>OH</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>8</td>
<td>9</td>
<td>3</td>
<td>20</td>
</tr>
<tr>
<td>Male:female</td>
<td>4:4</td>
<td>6:3</td>
<td>3:0</td>
<td>13:7</td>
</tr>
<tr>
<td>Age mean (range) years</td>
<td>64 (53-73)</td>
<td>65 (49-73)</td>
<td>66 (58-75)</td>
<td>65 (50-75)</td>
</tr>
</tbody>
</table>
Table 5. Systemic diseases and systemic medications and mean systemic blood pressure at fluorescein angiography study 3 and 4

<table>
<thead>
<tr>
<th>Systemic disease</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>none n=9</td>
<td></td>
</tr>
<tr>
<td>prostatic cancer n=1</td>
<td></td>
</tr>
<tr>
<td>systemic hypertension n=5</td>
<td></td>
</tr>
<tr>
<td>osteoporosis n=1</td>
<td></td>
</tr>
<tr>
<td>systemic hypertension and angina pectoris n=1</td>
<td></td>
</tr>
<tr>
<td>systemic hypertension and non-allergic asthma n=1</td>
<td></td>
</tr>
<tr>
<td>systemic hypertension and chronic bronchitis n=1</td>
<td></td>
</tr>
<tr>
<td>diaphragmal hernia n=1</td>
<td></td>
</tr>
<tr>
<td>Acetylsalicylic acid and Furosemide n=1</td>
<td></td>
</tr>
<tr>
<td>Acetylsalicylic acid n=1</td>
<td></td>
</tr>
<tr>
<td>Omeprazole n=1</td>
<td></td>
</tr>
<tr>
<td>Enalapril n=1</td>
<td></td>
</tr>
<tr>
<td>Bendroflumethiazide and inhalation Sodium Chromoglicate n=1</td>
<td></td>
</tr>
<tr>
<td>Hydrochlorthiazide n=4</td>
<td></td>
</tr>
<tr>
<td>Hydrochlorthiazide and Omeprazole n=1</td>
<td></td>
</tr>
<tr>
<td>Biphosphonates and Cholecalciferol n=1</td>
<td></td>
</tr>
</tbody>
</table>

Mean systemic blood pressure mmHg 153/93
Study 1: Analysis of retinal mean transit time from fluorescein angiography in human eyes: normal values and reproducibility

Two angiograms of the same eye were recorded in 25 healthy individuals. We were able to analyse both angiograms in 18 of these individuals and the results of these 36 angiograms are presented.

Failure to analyse one of the angiograms in the remaining seven subjects was attributable to one of two factors: either the recording time was too short to capture the whole dye curve (in five subjects) or marked rotation of the eye prevented alignment of at least one of the angiograms (in two subjects).

Mean values of $MTT_{IR}$, $MTT_{SLOPE}$ and AVP at the first measurement were 3.22, 4.88 and 1.46 seconds respectively.

Mean values of $MTT_{IR}$, $MTT_{SLOPE}$ and AVP at the second measurement were 3.0, 4.94 and 1.45 seconds respectively.

Inter- and intra-individual variability for the three analyses is presented in Table 6. The lowest inter-individual variability was observed with $MTT_{IR}$, while intra-individual variability was similar for $MTT_{IR}$ and AVP.

Table 6. Coefficient of variation for differences divided by mean of the two means.

<table>
<thead>
<tr>
<th></th>
<th>$MTT_{IR}$</th>
<th>$MTT_{SLOPE}$</th>
<th>AVP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coefficient of variation inter</td>
<td>24.3 %</td>
<td>40.1 %</td>
<td>39.2 %</td>
</tr>
<tr>
<td>Coefficient of variation intra</td>
<td>22.2 %</td>
<td>59.8 %</td>
<td>28.2 %</td>
</tr>
</tbody>
</table>

The best correlation was between $MTT_{IR}$ and $MTT_{SLOPE}$, $r=0.74$, $p<0.0005$. The correlation between $MTT_{IR}$ and AVP, $r=0.73$, $p<0.001$. The correlation between $MTT_{SLOPE}$ and AVP was weak, $r=0.52$, $p<0.04$.

Detection of an increase of 25% with a power of 80% requires groups of 14, 43 and 40 individuals for the three techniques, respectively.
Study 2: Retinal mean transit time in patients with primary open-angle glaucoma and normal tension glaucoma

MTT_{IR} could be analyzed in all 40 angiograms. The MTT_{IR} was 4.8 ± 1.4 seconds in the entire group. Mean (SD) was 5.03 (1.48) sec in eyes with POAG and 4.69 (1.44) sec in eyes with NTG. The difference was not statistically significant. There was a weak but significant correlation between MD and MTT_{IR}; MTT_{IR} = 4.12 – 0.08 * MD. (r=-0.49. p = 0.0013).

Study 3: The effect of elevated intraocular pressure on the retinal circulation in open-angle glaucoma and ocular hypertension. A study on retinal mean transit time

The average IOP in study eyes was 35 mmHg (range 22-62 mmHg). After one to two weeks on latanoprost the average IOP in 19 study eyes was 21 mmHg (the patient with IOP 62 mmHg on visit one withdrew from the study).

Fluorescein angiograms were recorded from 20 eyes with elevated IOP at the first visit. Two angiograms from two different patients, both from the eye with elevated IOP, could not be analyzed due to insufficient quality of the angiograms. The arterial curves did not reach a well-defined peak value of fluorescein intensity. One patient was withdrawn at the first visit and no angiogram was performed for the control eye in this patient. Thus 17 fluorescein angiograms from the eye with newly detected POAG or OH, one before and one after topical latanoprost were analyzed. Nineteen fluorescein angiograms of the control eye were analyzed. In one of these angiograms AVP could not be determined due to irregularities in the dye curves.

The average MD was -5.1 dB in the study eye and -1.5 dB in the control eye (p-value 0.009). All control eyes had glaucoma hemifield test (GHT) within normal limits, among the study eyes 17 had GHT outside normal limits and 3 inside normal limits (OH).

The neuroretinal rim area (NRA) measured with the HRT was 1.16 in the study eye and 1.43 in the control eye (p-value 0.005). There was a significant relation between NRA and MD.

There was a statistically significant correlation between MTT_{IR} and ocular perfusion pressure before IOP reduction in the study eye. No significant correlation between MTT_{IR} and systemic BP or MTT_{IR} and IOP was found.

A statistically significant difference between MTT_{IR} before and after treatment, as well as between MTT_{IR} before treatment in the study eye and the control eye was observed. No statistically significant difference was
found in AVP before and after IOP reduction or between AVP in the eye with elevated IOP and the control eye, Table 7.

Table 7. MTT_{IR} and AVP in study eye, before and after treatment, and in control eye.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Study eye before treatment Mean±SD (n)</th>
<th>Study eye after treatment Mean±SD (n)</th>
<th>Control eye Mean±SD (n)</th>
<th>p-value for difference study eye before and after (n)</th>
<th>p-value for difference study eye before treatment and control eye (n)</th>
<th>p-value for difference study eye after treatment and control eye (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTT_{IR}</td>
<td>4.77 ± 2.08 (19)</td>
<td>4.04 ± 1.67 (18)</td>
<td>3.52 ± 0.85 (18)</td>
<td>0.045</td>
<td>0.040</td>
<td>0.27</td>
</tr>
<tr>
<td>AVP</td>
<td>2.19 ± 0.86 (19)</td>
<td>1.87 ± 0.59 (18)</td>
<td>1.81 ± 0.37 (18)</td>
<td>0.14</td>
<td>0.13</td>
<td>0.68</td>
</tr>
</tbody>
</table>

A statistically significant correlation between MTT_{IR} and visual field damage, expressed as MD, was seen in the study eye after IOP reduction but not before IOP reduction, p-value 0.023. There was no significant correlation between AVP and MD either before or after IOP reduction.

Study 4: The optic nerve head in open-angle glaucoma and ocular hypertension. A study with fluorescein and ICG angiography

All patients with POAG or PEX had glaucoma hemifield test (GHT) outside normal borders while all eyes with OH had GHT within normal limits. The mean age was the same in the three groups. The mean deviation (MD) ranged from –3.7 to –28.1 dB (mean –6.2 dB) in eyes with POAG and from –5.2 to –29.4 dB (mean –8.2 dB) in eyes with PEX. Mean NRA was smaller in eyes with PEX compared to eyes with POAG, 0.92 vs 1.35 mm² (p = 0.06) and significantly smaller in study eyes than in fellow eyes, 1.16 vs 1.43 (p = 0.005).

There was a highly significant difference in IOP between the two eyes with a mean IOP in the study eye of 35 mm Hg, range 22 to 62 mm Hg. After treatment the study eyes had an IOP within or close to the normal range.

The shape of the relationship between MD and NRA was distinctly curvilinear and for a statistical analysis we choose to analyse the linear regression of MD on log NRA. Global values of MD and NRA and values for the upper field against the lower half of the disc and the lower field against the upper half of the optic disc were analysed. The two examinations showed good correlation with r² values of at least 0.67 and p-values 0.00002 or less.
Table 8 presents the peripapillary atrophy of the study eye and the fellow eye as well as MD. A definite peripapillary atrophy was observed in all three cases with MD > -20 dB and in ten of the sixteen cases with MD < -20 dB. In the fellow eyes nine out of eighteen had a definite peripapillary atrophy. In all eyes, study and fellow eyes, there was a lack of fluorescein filling in the corresponding area of the β zone, consistent with a drop-out of chorio-capillary vessels. In general fluorescein angiography (FAI) and ICG identified the same hypofluorescent areas, but in several angiograms peripapillary hypofluorescence was observed with ICG but difficult to identify with FAI. Red free and HRT pictures could identify the peripapillary atrophy as well.

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>Definitive peripapillary atrophy</th>
<th>Suspected peripapillary atrophy</th>
<th>No peripapillary atrophy</th>
</tr>
</thead>
<tbody>
<tr>
<td>MD&lt;-10 dB</td>
<td>12</td>
<td>8</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>MD -10 –20 dB</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>MD &gt; -20 dB</td>
<td>3</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEX</td>
<td>8</td>
<td>5</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>POAG</td>
<td>9</td>
<td>6</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>OH</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fellow eyes</td>
<td>19</td>
<td>9</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

The number of low-fluorescent pixels in the upper and lower half of the optic disc angiograms was determined twice with FAI (before and after reduction of IOP) and once with ICG (before treatment). There was no obvious effect on the distribution of low-fluorescent pixels by reducing the IOP, and the two angiograms can be used as a measure of the reproducibility of the technique. There was a highly significant correlation between the results obtained with FAI before and after treatment, $r^2$ of 0.86, for both the upper and the lower halves of the disc ($p < 0.000000$). Also, comparing the results obtained with FAI and ICG before treatment showed a strong correlation, with p-values of $<0.00002$ and $r^2$-values of 0.72-0.73 for the upper and lower disc halves.

The distribution of low-fluorescent pixels obtained by analysing the proportion of low-fluorescent pixels in the two halves of the optic disc was compared with the MD calculated from Humphrey perimetry for the upper and lower half of the visual fields. In this group of newly diagnosed patients with various levels of disc damage we found no significant correlation between function (MD) and number of low-fluorescent pixels in the corresponding half of the optic disc. However, there was a clear tendency towards better agreement between distribution of low-fluorescent pixels and field loss when eyes were ranked according to difference in MD between upper and lower visual field. This tendency, however, was not statistically significant ($p = 0.11$), Mann-Whitney U-test.
Calculation of MTT requires the reconstruction of the complete dye curves, from first appearance of the dye in the vessel until all dye has disappeared. Early recirculation of the dye distorts the downward slope so that this part of the curve has to be reconstructed by extrapolation from a semi-logarithmic plot. The short part of the down-slope that can be used sometimes makes the technique very inaccurate. A method where the whole curve up to recirculation is used to find the log-normal distribution function that optimally fits the first passage, has been used several times. AVP defined as the time difference between the first appearance of the dye in the artery and its nearby vein is another method of determining transit times from fluorescein angiograms. The advantage is that the analysis is to a large extent independent of the dye curves. The disadvantage is that it provides only a rough estimate of MTT as it estimates only the transit from artery to vein close to the optic disc, without taking into account transit in the peripheral part of the retinal vascular bed.

The advantage of MTTIR is that it is less dependent of the shape of the dye curves. This is important because the bolus might get distorted in many patients. The whole dye curve is used with the MTTIR technique. MTTIR is a defined physiological parameter. Both inter- and intra-individual variability were considerably smaller for MTTIR than for MTTslope.

In a clinical material with primary open-angle glaucoma and normal tension-glaucoma patients all 40 angiograms could be analyzed. There was no difference in MTTIR between the POAG and the NTG groups.

A correlation between the visual field defect, expressed as MD, and MTTIR was found, with longer transit times in eyes with marked visual field damage. The correlation coefficient, -0.49, indicates that other factors, including measurement errors, also are important then MD for MTTIR; the level of visual field damage will only explain about 24% of the variation in MTTIR.

Loss of neuronal tissue in glaucoma seems to be combined with an effect on the retinal circulation and the effect is similar in eyes with NTG and in eyes with POAG. Whether changes in MTTIR are primary or secondary to loss of neuronal tissue cannot be established from this study, and a reduction in retinal blood flow secondary to loss of neuronal tissue is a possible explanation.
In the study with newly detected POAG or OH we recorded fluorescein angiograms before and after initiation of treatment to reduce the IOP level and we compared the results with the normal control eye. Although the visual fields and optic discs in three eyes were normal they all had pronounced increases in IOP (IOP 31, 33 and 36 mmHg) and were included in the study since the can provide information on the effect on MTT IR of a marked reduction in IOP.

There was a statistically significant difference in MTT IR between the study eyes and the control eyes before treatment was initiated, and treatment tended to normalize MTT IR in study eyes. As MTT IR depends on ocular blood flow and vascular volume it is not possible to exclude that changes in vascular volume have contributed, i.e. that increased IOP increases vascular volume, but is clearly quite possible that blood flow is reduced in eyes with high IOP. In normal eyes retinal blood flow is autoregulated and moderate increases in IOP are not expected to have an effect on retinal blood flow.

The longer MTT IR in the study eye before treatment, compared to the control eye, could obviously be due to either the high IOP or the disease. The tendency to more normal values after reduction of IOP argues that at least part of the prolonged MTT IR before treatment is due to the high IOP. Thus patients with newly diagnosed POAG had close to normal retinal circulation when IOP was normalized but the retinal circulation was clearly affected by an increased IOP.

The degree of damage was evaluated by visual fields (MD) and optic disc morphology (NRA). The mean deviation of the superior and inferior halves of the visual field was estimated by averaging the recorded light sensitivity for those points. When NRA was plotted against MD there was a distinct curvilinear relationship and therefore we analysed the regression of MD on log NRA. There was strong a correlation between MD and log NRA whether global values were used or when the corresponding upper or lower halves of the fields and discs were compared. The significant relationships we observed is largely due to eyes with marked field loss, MD worse than −15 dB.

The results observed reveals more effects on structure (NRA) then on function (MD) in the early stages of glaucoma. As pointed out by Reus and Lemij\textsuperscript{107} part of the explanation may be that light sensitivity is expressed in a decibel scale and reductions in light sensitivity will seem smaller in near normal fields than in fields with advanced damage.

ICG detects areas of peripapillary hypoperfusion when the capillaries of the FAI pictures sometimes hide the underlying hypoperfused areas. ICG penetrates deeper into the layers of the choroid\textsuperscript{108, 109} and reveals areas of peripapillary hypoperfusion even when not visible on the FAI picture. Red free pictures and HRT pictures gave a good picture of the peripapillary atrophy together with the ICG picture.
Conclusions

In study 1 we used a new technique, MTT\textsubscript{IR}, to examine retinal mean transit time in human healthy eyes from fluorescein angiograms. The technique allows analysis of badly defined fluorescein dye curves. Normal values and reproducibility were determined in healthy eyes. The reproducibility achieved with MTT\textsubscript{IR} analysis was better than analysis of the angiograms with the conventional MTT\textsubscript{SLOPE} technique. Retinal MTT is defined as the volume of the retinal vascular bed divided by the blood flow through the retina and thus provides information on the retinal circulation. The analysis used in the present study allowed analysis of most angiograms recorded with the same routines that are used in clinical work.

There was no difference in MTT between patients with primary open-angle glaucoma and patients with normal tension glaucoma. There is a correlation between MTT and visual field damage, both in patients with newly detected and in patients with previously known open-angle glaucoma. It seems reasonable to conclude that the relationship between MD and MTT\textsubscript{IR} reflects a reduction in retinal blood flow with increased axon loss rather than an increase in vascular volume.

In eyes with markedly increased intraocular pressure MTT is prolonged compared to the fellow control eye but MTT is reduced when the intraocular pressure is reduced to normal or near-normal levels.

A likely explanation is that the autoregulatory capacity of the retinal vascular bed is insufficient to maintain normal retinal circulation in the IOP range observed in a group of unselected, newly diagnosed patients.

There is a significant correlation between loss of neuroretinal rim area and visual field defects. Peripapillary atrophy is common in eyes with glaucoma, whether they have pseudoexfoliations or not, but they were also common, although less pronounced, in the non-glaucomatous fellow eyes. Loss of peripapillary choriocapillaris is seen in all atrophic areas, but can be better identified with ICG than with FAI. In digitised frames from the angiographies the pixel with the lowest grey scale values tend to be more frequent in the most damaged part of the optic disc.
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Sammanfattning på svenska


Olika indirekta tekniker att undersöka näthinnans cirkulation finns redovisade i den vetenskapliga litteraturen. Med idag finns det ingen teknik som direkt mäter det totala blodflödet i näthinnan eller synnerven i det mänskliga ögat.

I våra studier har vi utvecklat en teknik som mäter hur lång tid ett kontrastämne tillbringar i näthinnans kärlbädd. I den första studien undersöktes 18 friska frivilliga för att erhålla ett normalvärde och för att undersöka metodens tillförlitlighet.

Därefter har 40 patienter med tidigare diagnostiserade öppenvinkel glaukom och lågtrycks glaukom samt 20 nyupptäckta, obehandlade patienter med ensidig ökning av ögontrycket undersökt. Glaukom är en ögonsjukdom som ger upphov till förlust av nervtrådar i synnerven och därmed följande bortfall av delar av synfältet.

I gruppen med tidigare diagnostiserade öppen-vinkel glaukom fann vi en korrelation mellan längre cirkulationstid i näthinnans kärlbädd och synfältsbortfall. Det förelåg ingen skillnad mellan öppen-vinkel glaukom och lågtrycks glaukom i cirkulationstid.

I den nyupptäckta gruppen med ensidigt obehandlat öppen-vinkel glaukom fann vi en skillnad i cirkulationstid före och efter sänkning av ögontrycket med latanoprost ögondroppe samt en skillnad mellan ögat med tryckstegning och det normala ögat.

Cirkulationen i synnervshuvudet har undersöpts med angiografi med två olika kontrastämnen, natrium fluorescein och indocyanin grön i gruppen med nyupptäckta öppenvinkel glaukom. Det fanns en tendens till sämre kontrastfyllnad i den mest skadade delen av synnervshuvudet. En stark korrelation sågs också mellan utbredningen av synnervsskadad, bestämd med laser tomografi, och synfältsbortfall.
References


A doctoral dissertation from the Faculty of Medicine, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine. (Prior to January, 2005, the series was published under the title “Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine”.)