The impact of ocular blood flow in glaucoma

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Abstract

Two principal theories for the pathogenesis of glaucomatous optic neuropathy (GON) have been described—a mechanical and a vascular theory. Both have been defended by various research groups over the past 150 years. According to the mechanical theory, increased intraocular pressure (IOP) causes stretching of the laminar beams and damage to retinal ganglion cell axons. The vascular theory of glaucoma considers GON as a consequence of insufficient blood supply due to either increased IOP or other risk factors reducing ocular blood flow (OBF). A number of conditions such as congenital glaucoma, angle-closure glaucoma or secondary glaucomas clearly show that increased IOP is sufficient to lead to GON. However, a number of observations such as the existence of normal-tension glaucoma cannot be satisfactorily explained by a pressure theory alone. Indeed, the vast majority of published studies dealing with blood flow report a reduced ocular perfusion in glaucoma patients compared with normal subjects. The fact that the reduction of OBF often precedes the damage and blood flow can also be reduced in other parts of the body of glaucoma patients, indicate that the hemodynamic alterations may at least partially be primary. The major cause of this reduction is not atherosclerosis, but rather a vascular dysregulation, leading to both low perfusion pressure and insufficient autoregulation. This in turn may lead to unstable ocular perfusion and thereby to ischemia and reperfusion damage. This review discusses the potential role of OBF in glaucoma and how a disturbance of OBF could increase the optic nerve’s sensitivity to IOP. © 2002 Elsevier Science Ltd. All rights reserved.

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1. Introduction

The term glaucoma is used to cover a wide range of diseases, traditionally including any and all conditions associated with increased intraocular pressure (IOP). However, by virtue of the fact that not all patients with glaucomatous optic neuropathy (GON) have increased IOP, and not all patients with increased IOP suffer from GON, new definitions were introduced. Some authors equate glaucoma with GON (van Buskirk and Cioffi, 1992). In practice it has proven useful to speak about glaucoma patients if they present either with elevated IOP, GON or both (Flammer, 2001a). Whereas a number of indisputable risk factors (RFs), including elevated IOP, have been described, the pathogenesis leading to GON remains poorly understood.
In the past, two theories were presented, the mechanical and the vascular (Fechtner and Weinreb, 1994). The mechanical theory supposes that GON is a direct consequence of IOP, damaging the laminar cribrosa and neural axons (Yan et al., 1994). The vascular theory considers GON as a consequence of insufficient blood supply due to either increased IOP or other RFs reducing ocular blood flow (OBF) (Flammer, 1994). The differentiation between mechanical and vascular theories had little clinical impact so far, as IOP reduction was the only intervention available for treatment of glaucoma. However the introduction of new therapeutic options, such as pharmacological blood flow modulation, call for a more detailed description of the RFs and a better understanding of GON pathogenesis. The purpose of this review is to summarize some of the present knowledge of the pathogenesis of GON, with special emphasis on the role of OBF.

2. Historical review

Elevation of IOP as a distinct sign of ocular disease recognizable by undue eyeball resistance to indentation by the physician’s finger, was first clearly mentioned in the breviary of the itinerant English oculist Bannister in 1626 in a book titled “A worthy treatise of the eyes” (Shaffer, 1996). In the early 1800s firmness of the eyeball was generally accepted as a distinct disease entity, which McKenzie described as glaucoma (MacKenzie, 1830).

In 1851, the invention of the ophthalmoscope by Hermann von Helmholtz enabled ophthalmologists to observe the optic nerve head (ONH) directly. Initially the ONH of glaucoma patients was apprised as prominent, but in mid-1850s von Graefe, Weber and Müller independently and correctly described it as an excavation and linked it to elevated IOP. This relationship was so evident that von Graefe considered increased IOP as the “essence” of glaucoma (von Graefe, 1857). However, as early as 1858, Jaeger proposed that GON might have other intrinsic causes, not necessarily IOP related (Jaeger, 1858). Smith in 1885 suggested the involvement of both mechanical and vascular factors (Smith, 1885). Since then, the mechanical and vascular theories have been defended by various research groups (Fechtner and Weinreb, 1994).

As techniques for measuring IOP became more reliable and accessible, tonometry became the main method for assessing glaucoma, to the detriment of other RFs (van Buskirk and Cioffi, 1992). In the early 1980s it was proposed that both the mechanical and vascular theories might be correct and that in most cases the two mechanisms might in fact act synergistically (Flammer, 1985b). With the advent of modern techniques to quantify glaucomatous damage, measure OBF and, importantly, through the development of modern molecular biology, new insights in the disease process have been gained and will most probably enable us in the near future to understand the cascade of events on a cellular and molecular level.

3. The role of IOP in glaucoma

A number of conditions such as congenital glaucoma, angle-closure glaucoma or secondary glaucomas clearly show that increased IOP is sufficient to lead to GON. According to the mechanical theory an increased pressure leads to elongation, stretching and collapse of the laminar beams and their posterial displacement (bowing). The axons of the retinal ganglion cells become damaged either directly, by increased pressure and pressure gradient, or indirectly, by tissue deformation. The axoplasmatic transport is impeded which may ultimately induce cell death, for example due to a lack of trophic factors. According to the vascular theory, increased IOP may also disturb blood supply leading to tissue ischemia and finally cell death.

The role of IOP is, even in NTG patients, further supported by the beneficial effect of IOP lowering treatment (Collaborative Normal-Tension Glaucoma Study Group, 1998). Both experimental as well as clinical intervention studies have proven the role of IOP and the benefits of IOP lowering treatment (The AGIS Investigators, 2000). Although a marked IOP reduction improved the prognosis of patients, it cannot stop progression in all patients.

The assumption that IOP is the only relevant RF is being increasingly challenged (Flammer, 1995). The existence of normal-tension glaucoma (NTG) on one hand and patients with ocular hypertension (increased IOP without recognizable damage) on the other, indicate that other factors might also be involved in the pathogenesis of GON either directly or indirectly by rendering the eye more sensitive to IOP. We must ask the questions: why is it that at a given pressure level, e.g. 22 mm Hg, the majority of people will never suffer GON, while others do? Furthermore, why is GON progression only weakly related to IOP levels (Niesel and Flammer, 1980; Chauhan and Drance, 1992; Weber et al., 1993; Martinez-Bello et al., 2000)? Finally pressure reduction, although significantly improving prognosis does not avoid damage in all patients (Stewart et al., 2000).

Although men and women have similar IOPs, NTG incidence in women is twice that of men’s (Orğul et al., 1995a,b). Patients of African descent not only have higher IOPs on average (Sommer et al., 1991), but also have a greater likelihood of developing GON at given IOP levels (Leske et al., 1995; Tielsch et al., 1995). In Japan, at least half of all glaucoma patients have NTG (Araie et al., 1994). Although in Japan GON incidence.
increases with age at a similar rate as in America or Europe (Shiose, 1990a), on average IOP diminishes with advancing age. However it should be noted that despite this, Japanese patients with elevated IOP, like their Western counterparts, are more likely to have progressive GON than those with normal IOP (Shiose, 1990b). All these observations cannot be satisfactorily explained by the pressure theory alone, indicating that increased IOP, although clearly sufficient, is not necessary for the development of GON (Flammer and Orgül, 1998).

Other established RFs include myopia, genetic predisposition, gender, race and possibly the presence of autoimmune diseases (Leske et al., 1995; Morgan and Drance, 1975; Wilson et al., 1987; Galassi et al., 1998; Reynolds, 1977; Katz and Sommer, 1988; Cartwright et al., 1992). Of special interest are vascular and, potentially, rheological factors (Flammer and Orgül, 1998; Hamard et al., 1994). These vascular RFs are not only often related to GON, but are also potential therapeutic targets. Before we discuss the literature on OBF, we will briefly review the anatomy and physiology of OBF, followed by the methodology to quantify it.

4. Anatomy and physiology of OBF

The anatomy and physiology of OBF, including the ONH blood flow has been reviewed previously (Flammer and Orgül, 1998; Hayreh, 1996; Bill and Nilsson, 1985; Buechi, 1995). In order to enable a better appreciation of the vascular insufficiency occurring in glaucoma, a summary of the anatomy and physiology of OBF is presented here.

Ocular circulation is complex, because of the necessity to supply different ocular structures with nutrients without interfering with the visual pathway. OBF is highly regulated in order to adapt to changing metabolic needs during changing visual function, to compensate for varying perfusion pressures and finally to keep the temperature at the back of the eye constant (Alm and Bill, 1973; Delaey and Van De Voorde, 2000; Alm, 1998).

Retinal vessels are supplied by the central retinal artery, which in turn is a branch of the ophthalmic artery (Fig. 1) (Olver, 1998). The anatomy and physiology of the retinal circulation resembles the brain circulation with the exception that the retinal circulation has no autonomic innervation. Retinal circulation is characterized by a low level of flow and high level of oxygen extraction. The presence of endothelial tight junctions results in a blood–retinal barrier, similar to the blood–brain barrier. Circulation is autoregulated, meaning that within a certain range flow is independent of perfusion pressure (Grunwald et al., 1982; Pournaras, 1995; Riva et al., 1981; Dumskyj et al., 1996; Schulte et al., 1996). Beside myogenic regulation, endothelial cells play a major role in local regulation (Haefliger et al., 1992; Meyer et al., 1993; Haefliger et al., 1994; Meyer et al., 1995; Haefliger et al., 2001; Orgül et al, 1999). Factors involved in this regulation are the partial pressure of oxygen (Fig. 2) and carbon dioxide, circulating and locally produced hormones like angiotensin-II and local metabolites like adenosine diphosphate (Stefansson et al., 1988).

In contrast, choroidal circulation is characterized by very high flow and low oxygen extraction. Choroidal blood flow accounts for 85% of the total blood flow in the eye. Besides supplying the retina with nutrients, regulation of choroidal circulation seems to be important for maintaining temperature and volume in the eye (Flugel et al., 1994; Flugel-Koch et al., 1996). The choroid is supplied by the posterior ciliary arteries, branching from the ophthalmic artery. The endothelium of the choriocapillaries are fenestrated. Poor autoregulation renders choroidal blood flow more dependent on perfusion pressure (Delaey and Van De Voorde, 2000;
Geiser et al., 2000; Kiel, 1999; Findl et al., 1997; Riva et al., 1997b). Like extraocular vessels, the choroid has a rich autonomic innervation (Fig. 3) (Chou et al., 2000; May et al., 1997; Steinle et al., 2000). Sympathetic nerves reach the eye from the superior cervical ganglion following the path of the blood vessels, while parasympathetic nerves reach the eye through the ocular motor nerve, facial nerve and through the ophthalmic and maxillary division of the trigeminal nerve. A number of neural transmitters are involved, like norepinephrine, acetylcholine, nitric oxide, vasoactive intestinal peptide and others (Haefliger et al., 1999a, b; Koss, 1999; Schrodl et al., 2001).

Blood supply to the ONH is very unique by any standard (Figs. 4 and 5) (Olver, 1998). The superficial layer of the ONH receives its blood supply via small branches of the central retinal artery. The prelaminar region, a small area anterior to the laminar cribrosa, is mainly supplied by branches from recurrent choroid arterioles and the short posterior ciliary arteries (Hayreh, 1996; Hayreh, 2001; Ernest, 1976; Lieberman et al., 1976; Onda et al., 1995). Venous drainage of the ONH is through the central retinal vein. The ONH seems to be the only part of the central nervous system which has no proper blood–brain barrier, with the capillaries lacking blood–brain barrier properties (Hofman et al., 2001). In addition some diffusion from the surrounding choroid into the ONH is possible. This makes ONH circulation especially sensitive to circulating molecules like endothelin-1 and angiotensin II (Flammer and Organ, 1998). The autoregulation seems to be less efficient than in the retina but better than in the choroid (Pournaras and Riva, 2000; Movaffaghy et al., 1998; Riva et al., 1997a, b; Shonat et al., 1992).

The question of whether OBF can be measured in patients and, indeed, whether it is altered in glaucoma remains. If altered, we need to consider whether this is a secondary effect, caused either by increased IOP or the presence of GON or whether changes in OBF are an independent primary factor. Finally, should it be primary, what could be its cause?
5. Techniques for evaluating OBF

Many different methods are used to visualize and measure directly or calculate indirectly in vivo OBF. Although there have been many advances in techniques over the past 20 years, there is still no single method that can provide all the relevant information in one reading. The fact that the different methods available also measure different aspects of ocular perfusion (for example flow velocity or vessel size, etc.) and at different locations in the eye makes direct comparisons between techniques all but easy.

Nevertheless the development of newer techniques and their correct use combined with a careful analysis and consideration of the results, provides the potential for assessing blood flow in humans. As the techniques for measuring OBF have been described previously by many different authors (Shiose, 1990b; Flammer and Orgül, 1998; Pillunat, 1999; Pillunat et al., 1999; Zarfati et al., 2000; Harris et al., 1999; Williamson and Harris, 1994), only the main advantages and drawbacks will be outlined here and summarized in Table 1.

5.1. Pulsatile ocular blood flow

Arterial blood flow to the eye varies with the heart cycle. Correspondingly the volume (especially of the choroid) and the IOP are highest during systole and lowest during diastole. To estimate the pulsatile component of OBF, different methods are used (Krackau, 1995).

The Langham OBF system measures the pulse wave of the rhythmic change in IOP during the cardiac cycle (Kergoat, 1997; Langham, 1994; Langham et al., 1989; Silver et al., 1989). The instrument consists of a modified 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 blood flow. This technique requires several assumptions to be made with inaccuracies occurring if the rigidity or the size of the eye differs (Migdal, 1999). Gender also has an influence independent of other hemodynamic parameters (Gekkieva et al., 2001).

Another method quantifies choroidal pulsation by means of interferometry (Schmetterer et al., 2000; Wolzt et al., 1995). Both methods are convenient for studies of intra-individual blood flow changes (e.g. before and after medication) rather than comparing different groups (e.g. glaucoma patients vs. normal subjects). Ocular pulsation can also be monitored with the help of the so-called SmartLens® (Dekker et al., 1998–99). Furthermore, measurement of arterial oxygen tension by means of pulse oximetry is also based on the pulsation (de Kock et al., 1993).

5.2. Angiography

Angiography visualizes the passage of fluorescent dye through ocular vessels. In fluorescent angiography, fluorescein is injected into the cubital vein. 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 (Fig. 6). Therefore 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 much more challenging, as the endothelium of the choriocapillaries have ample partially permeable openings for molecules the size of fluorescein. Thus, indocyanine green is used for better visualization of choroidal circulation (Zarfati et al., 2000; Prünte and Niesel, 1988; Lambrou, 1999; Arend et al., 1999). This is a dye which absorbs mid-infrared light, and once injected binds rapidly and almost completely to certain plasma proteins, preventing the dye from leaving the fenestrated endothelium of the choriocapillaries (Fig. 7).

Dye passage through the eye is recorded by photo or video. By improving the imaging technique with the help
### Table 1
Summary of OBF measurement techniques and the parameters used

<table>
<thead>
<tr>
<th>Method</th>
<th>Vascular bed</th>
<th>Parameter measured</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>The Langham OBF system Laser interferometry</td>
<td>Choroid, optic disc</td>
<td>IOP</td>
<td>Pulsatile choroidal blood flow</td>
</tr>
<tr>
<td></td>
<td>Choroid, optic disc</td>
<td>Varying distance between cornea and fundus</td>
<td>Pulsatile blood flow at the ocular fundus</td>
</tr>
<tr>
<td>Smart lens</td>
<td>Retina</td>
<td>IOP</td>
<td>Allows to assess the effect of IOP on retinal blood flow</td>
</tr>
<tr>
<td>Fluorescein angiography</td>
<td>Retina</td>
<td>Velocity of small intravascular substances</td>
<td>Velocity of retinal blood flow</td>
</tr>
<tr>
<td>Indocyanine green angiography</td>
<td>Choroid</td>
<td>Velocity of intravascular proteins</td>
<td>Velocity of choroidal blood flow</td>
</tr>
<tr>
<td>Laser Doppler velocimetry</td>
<td>Retina</td>
<td>Blood flow velocity in retinal vessels</td>
<td>Blood flow velocity in large retinal vessels</td>
</tr>
<tr>
<td>Laser Doppler flowmetry</td>
<td>Retina, optic disc</td>
<td>Effect of capillary blood flow on retinal surface</td>
<td>Capillary blood flow in the choroid or the optic disc</td>
</tr>
<tr>
<td>Heidelberg retina flowmeter</td>
<td>Retina, optic disc</td>
<td>Effect of capillary blood flow on retinal surface as well as on laser light</td>
<td>Capillary blood flow in the innermost layers of the retina or the optic disc</td>
</tr>
<tr>
<td>Laser speckle tissue blood flow analysis</td>
<td>Retina, optic disc</td>
<td>Effect of varying surface irregularities on laser light</td>
<td>Capillary blood flow in the innermost layers of the retina or the optic disc</td>
</tr>
<tr>
<td>Blue field entoptic technique</td>
<td>Foveal retina</td>
<td>Entoptic phenomenon produced by leucocytes</td>
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<tr>
<td>Retinal vessel analyzer</td>
<td>Retina</td>
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<tr>
<td>Corneal temperature</td>
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<tr>
<td>Nailfold capillary microscopy</td>
<td>Nailfold capillaries</td>
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<tr>
<td>Peripheral laser Doppler flowmetry</td>
<td>Peripheral vessels precapillaris, capillaries and shunt-vessels of the skin</td>
<td>Effect of capillary blood flow on laser light</td>
<td>Capillary blood flow in the skin</td>
</tr>
</tbody>
</table>

*Fig. 6. Fluorescent angiography: visualisation of the optic nerve head and retina perfusion.*

*Fig. 7. Picture taken during indocyanine green angiography of the papillary and peripapillary areas.*
of a scanning laser ophthalmoscope and a frame-by-frame analysis, some quantification is possible (i.e. arterial-venous passage time) (Greve et al., 1999). Although passage time may not be highly correlated with OBF (Tomic et al., 2001), it gives interesting information on ocular perfusion.

5.3. Laser Doppler techniques

The Doppler effect was first described by the Austrian physicist Christian Doppler in 1842. The Doppler effect describes the frequency shift that a light wave undergoes when emitted from an object which is moving away from or towards an observer (Fig. 8) (Bebie, 1995). Correspondingly, the Doppler effect applies to acoustic waves. Today, with lasers providing optical light waves of extreme purity, it is possible to detect Doppler shifts with very high resolution. Measurement of red cell blood velocity using this technique was first described in 1972 by Riva and co-workers (Riva et al., 1972; Riva et al., 1982). The laser Doppler principle can be used in different ways.

5.3.1. Laser Doppler velocimetry

Laser Doppler velocimetry (LDV) measures blood flow velocity (Logean et al., 1997; Michelson and Harazny, 1997; Logean et al., 2000). In order to do this, a laser beam is directed at a specific blood vessel. The blood flowing through this vessel causes a Doppler shift, or change in wavelength, allowing blood speed to be measured. This method provides fast, direct and quantitative measurement of absolute blood velocity (Mendel et al., 1993). By then measuring the vessel’s diameter, blood flow can be calculated. However, this provides only data on a single vessel and no information about the perfusion in the rest of the eye.

5.3.2. Laser Doppler flowmetry

Laser Doppler flowmetry (LDF) measures relative velocities, number of red blood cells and flux at a fixed spot (Riva et al., 1992). Unlike velocimetry, flowmetry measures the blood flow in capillary beds, with the laser directed to areas between larger vessels (Fig. 9) (Bonner and Nossal, 1990; Osusky et al., 1997; Osusky et al., 2000; Riva et al., 1992). One important issue is the depth of penetration by the light used, in order to know which component of the tissue is being studied (Koelle et al., 1993). Two wavelengths can be used, the first uses a visible light wavelength to measure the blood flow in the ONH and retina, while the second uses a longer wavelength, which penetrates the tissue further and measures choroidal blood flow underneath the macula (Riva et al., 1994a, b; Geiser et al., 1999; Straubhaar et al., 2000).

These methods provide information on capillary blood flow at a selected location over a fixed period of time in arbitrary units (Petrig and Riva, 1995; Petrig and Riva, 1999). Partializing out the influence of yield (parameter for tissue scattering properties) markedly improves the reproducibility of LDF (Gugleta et al., 2002). The technique’s main drawback is that measurements are limited to a small selected area and the exact volume of the tissue being measured is unknown. Therefore the technique does not allow easy comparison between individuals (Petrig and Riva, 1995), however it does remain useful for intra-individual comparisons.

5.3.3. Heidelberg Retina Flowmeter

The Heidelberg Retina Flowmeter (HRF) technique combines laser Doppler flowmetry with confocal scanning laser tomography (Fig. 10) (Bohdanecka et al., 1998; Lietz et al., 1998; Zinser, 1999; Chauhan and Smith, 1997; Griesser et al., 1999; Bohdanecka et al., 1999; Erb et al., 1999; Haefliger et al., 1999a, b; Lietz-Partzsch et al., 2001; Hayashi et al., 2000; Kagemann et al., 1998). The method is non-invasive and results are
rapidly obtained. It requires clear optical media and good fixation. It is highly sensitive to illumination changes (Kagemann et al., 2001), eye movement and measures blood flow within a relatively small velocity range. Furthermore, small changes in the sample window placement can yield large differences in flow measurement, leading to relatively limited reproducibility. Application of improved and sophisticated software may provide better results (Hosking et al., 2001; Jonescu-Cuypers et al., 2001; Michelson et al., 1998b).

5.4. Laser speckle phenomenon

The laser speckle phenomenon is an additional technique that has been developed to analyze OBF (Tamaki et al., 1995; Tomidokoro et al., 1995; Tomidokoro et al., 1998; Tamaki et al., 1994; Sugiyama et al., 1996; Yaoeda et al., 2000a; Yaoeda et al., 2000b; Tamaki et al., 2001). The technique is based on the principle that a random speckle pattern is created when laser light is shone on a matt surface such as living tissue. There are two basic ways of observing laser speckle. The first is to image the illuminated area. Whether this is done by the eye or by a camera, a granular speckle pattern will be seen on the surface of the object. This type of speckle is know as “image speckle”. The second way is to shine a narrow laser beam at the object and look at the scattered light falling on a screen some distance away. This type of speckle is called “far-field speckle”. Figs. 11 and 12 show the formation of the two types of speckle.

A typical speckle pattern is illustrated in Fig. 13. The effect is caused by light interference. Changes as small as half the wavelength of light (say 0.3 µm) in the relative positions of points in the illuminated area cause this pattern to fluctuate. In the case of blood flow, these fluctuations are related to the flow velocity.

Results from laser speckle measurements have been found to closely mirror those of LDF (Ruth, 1990). In fact, it can be shown that the speckle and Doppler techniques are really just two ways of looking at the same physical phenomenon (Briers, 1996; Briers, 2001).
By scanning, both the speckle and the Doppler techniques can provide an overall map of retinal and ONH blood flow. By scanning every 10 s, a picture of OBF over time can be produced for the selected area. However, there is a way to avoid the need to scan. A time-exposure photograph of a fluctuating speckle pattern will clearly show a reduction in speckle contrast. The faster the fluctuations, the greater will be the reduction in contrast. Hence by measuring the contrast of a time-integrated speckle pattern, information can be obtained about the frequency of the fluctuations and hence about the flow velocity. This has been developed into a technique known as laser speckle contrast analysis (Briers and Webster, 1996; Briers et al., 1999) and has been used recently in the monitoring of cerebral blood flow (Dunn et al., 2001).

Disadvantages of both speckle and Doppler techniques include the fact that blood flow is calculated and not measured directly.

5.5. Blue field entoptics

This technique is based on the flow of white blood cells, leukocytes, to assess retinal blood flow (Riva and Petrig, 1980; Scheiner et al., 1994; Arend et al., 1995; Petrig et al., 1997; Forcier et al., 1998; Zetlan et al., 1992; Salmenson et al., 1992; Grunwald et al., 1993; Kiss et al., 2000). Leukocyte velocity is usually pulsatile, as with the rest of the blood, matching the pumping heart with a velocity that increases at systole and decreases at diastole. The presence of leukocytes in the capillaries around the macula can be perceived by patients: when looking at diffuse blue light, they should be able to see small moving forms. It is assumed that these are leukocytes. Similar patterns are created by computer simulation, and subjects are asked to match the pictures on a computer screen with the one they can see while looking into a blue light. The pattern match can then be used to draw conclusions about retinal perifovea perfusion. However the conclusions that can be drawn from this data are limited as the quality of the data depends on the patient’s full cooperation. In addition, there are large variations between patients and only data from capillaries around the retinal fovea are provided.

5.6. Retinal vessel analyzer

The Zeiss retinal vessel analyzer is used to measure the diameter of the blood column in retinal blood vessels directly (Munch et al., 1995; Polak et al., 2000). This enables continuous monitoring of the vessel diameter, both as a function of location and time (Fig. 14) (Flammer, 2001a). At present little is know about the behavior of retinal vessel diameter in healthy and diseased eyes, however this technique may provide new information in the future (Jandrasits et al., 2001; Pache et al., 2002b).

5.7. Temperature

Although indirect, the measurement of temperature has long been a method for assessing blood flow in an
organ. Limbs, such as feet or hands, get colder if their blood flow is disturbed. This holds also true for the eye (Lychkovskii, 1967; Mapstone, 1968a; Mapstone, 1968b; Mapstone, 1969; Rysa and Sarvaranta, 1974; Alio and Padron, 1982; Zeiss, 1930). Corneal temperature can easily be measured (Flammer, 2001a; Kocak et al., 1999). It correlates significantly with blood flow in the ophthalmic artery (Gugleta et al., 1999), suggesting that OBF indeed influences corneal temperature (Morgan et al., 1999). The method can be used to study drug effects (Beano et al., 2001). Furthermore, corneal temperature correlates significantly with finger temperature, even after adjusting for environmental and tympanic temperature, suggesting a possible similarity between blood flow in the eye and finger (Girardin et al., 1999). The estimation of OBF, using this technique, is improved further if the re-warming of the eye is measured after it has been cooled briefly using an air-stream (Fig. 15). This non-invasive, easy and quick measurement provides only indirect information about the overall blood flow in the eye and, when there is a reduction, no information on the specific location of the disturbance is given (Flammer, 2001a).

5.8. Color Doppler imaging

The transcranial Doppler (Michelson et al., 1990; Rojanapongpun and Drance, 1993a; Rojanapongpun et al., 1993) has been increasingly replaced by Color Doppler Imaging (CDI). This is an ultrasound technique that combines B-scan gray scale imaging of tissue structure, color representation of blood flow based on Doppler shift and pulsed Doppler measurement of blood flow velocities. This method is well established in many different fields of medicine. In ophthalmology it is used to measure blood flow velocity in the retrobulbar vessels, using 5–7.5 MHz frequencies transducers (Lieb et al., 1990; Lieb et al., 1992; Galassi et al., 1992; Williamson et al., 1993; Pourcelot, 1974; Niwa et al., 1998; Sergott et al., 1994). Flow velocity data are plotted against time (Fig. 16). The peak and trough of the waves are then identified by the operator. From these points, peak systolic and end-diastolic velocities are measured. In addition the so-called pulsatility and resistivity indices can be calculated (Pourcelot, 1974; Evans et al., 1980). These vary from 0 to 1 with higher values supposedly indicating higher distal vascular resistance. Results can be obtained relatively easily from larger vessels such as the ophthalmic or central arteries, while obtaining data from the smaller posterior ciliary arteries remains difficult. The reliability and reproducibility of the results also depend on the experience of the user (Kaiser et al., 1996a,b; Kaiser et al., 1997a; Senn et al., 1996; Senn et al., 1999; Roff et al., 1999; Quaranta et al., 1997).
5.9. Periphereal blood flow

There are two reasons to measure peripheral blood flow (Haefeli et al., 1998) in glaucoma patients: (a) disturbances in peripheral blood flow would indicate that blood flow disturbance in glaucoma patients may not be confined to the eye; (b) there is some correlation between OBF and peripheral blood flow, especially in patients with vascular dysregulation (Gasser et al., 1999a).

To evaluate the peripheral microcirculation, two methods are of special interest in the present context: nailfold capillary microscopy (Fig. 17), (Mahler et al., 1989; Saner et al., 1987) and LDF (Gasser et al., 1992; Svedman et al., 1998; Gasser et al., 1989). The former measures velocity of capillary blood flow in the nailfolds of fingers. The latter measures bulk blood flow at any selected location on the skin, but does not distinguish between blood flow in the capillaries, pre-capillaries and shunt vessels (Flammer, 2001a). The correlation of the outcome of the two methods is therefore weak.

5.10. Perimetry

It may seem strange that perimetry is listed among the methods used to quantify OBF. Perimetry is used to assess the differential light sensitivity (DLS). The outcome fluctuates in normal eyes and this fluctuation is amplified in glaucoma patients. The fluctuation has both a short- and long-term component (Flammer et al., 1984c; Flammer, 1985a). The short-term component depends on factors like damage of the visual field or cooperation of the patient (Flammer et al., 1984a), while the long-term component (Flammer et al., 1983) seems to be, among other factors, influenced by ocular circulation. Experimental studies in healthy volunteers demonstrated that DLS is strongly correlated with blood–oxygen tension (Fig. 18) (Brandl and Lachennayr, 1994). Furthermore it has been demonstrated in glaucoma patients that the DLS threshold fluctuates parallel to IOP (Flammer et al., 1984b). In patients with vascular dysregulation, the improvement in visual fields, observed while undergoing vasoactive treatment such as with calcium channel blockers (Guthauser et al., 1988) or while breathing carbon dioxide (Pillunat et al., 1994), is correlated to the changes in blood flow observed in the periphery (Flammer, 1992). Likewise, the deterioration of visual fields after cold provocation also correlates with blood flow deterioration (Guthauser et al., 1988). Therefore a relatively quick (reversible) change of the visual field can be an indirect sign of a change in ocular perfusion (Flammer et al., 2001b).

5.11. Provocation tests

Blood flow in the eye of glaucoma patients might be normal under baseline conditions, but disturbed when challenged. Therefore a number of blood flow measurement techniques listed above are combined with provocation tests. Angiography (Ulrich et al., 1993), VEP (Zetlan et al., 1992; Kremmer et al., 1992; Pillunat et al., 1987), CDI (Harris et al., 1996), or even perimetry (Vanderburg and Drance; 1966; Scott and Morris, 1967) has been performed under artificially increased IOP. Microcirculation is often measured under cold provocation (Mahler et al., 1989; Saner et al., 1987; Flammer, 2001a), while choroidal blood flow is measured before and after stimulation of the sympathetic nervous system by hand grip (unpublished data). Visual fields can be tested before and after cold provocation (Guthauser et al., 1988) or the application of selective drugs (Gasser and Flammer, 1990) or breathing carbon dioxide (Pillunat et al., 1994). All these approaches provide additional information on OBF when challenged. In fact, abnormal responses to provocative tests only, may
be indicative of fluctuations in OBF between non-pathological and pathological states, depending on the patient’s environmental and emotional circumstances.

5.12. Experimental methods

Although not discussed here, it is worth noting that there are a number of additional techniques used to measure blood flow in the eye and optic nerve. These methods are not discussed here either because they can only be used in animals (e.g. the microsphere or casting techniques) or only in volunteers because they have not yet been developed for use in larger populations (e.g. blood flow quantification using MRI) (Prünste et al., 1995).

6. OBF in glaucoma

To summarize the findings of OBF studies in glaucoma is difficult for the following reasons: the authors use different techniques and therefore measuring different aspects of ocular circulation (Carter et al., 1990); they include glaucoma patients at different stages (e.g. early vs. late); different types of glaucoma are studied (e.g. NTG vs. high-tension glaucoma (HTG)); some include provocation tests, while others do not. Nevertheless, the vast majority of studies published find on average a reduced ocular perfusion in glaucoma patients. Blood flow decreases with increasing damage, however the reduction occurs in both early and late stages of glaucoma (Michelson et al., 1998a, b). The reduction in blood flow involves different parts of the eye, including the ONH (Harju and Vesti, 2001), choroid (Yin et al., 1997) and retinal circulation, as well as retrobulbar and even peripheral blood flow. Blood flow disturbances generally seem to be more pronounced in NTG than HTG (Drance et al., 2001). In most of the studies applying provocation tests, differences between glaucoma and normals were more pronounced under provocation (Flammer, 1995; Flammer et al., 1992), and finally blood flow reduction is more pronounced in progressive than non-progressive eyes (Yamazaki and Drance, 1997; Stewart et al., 2000). In the following section, some of the studies will be summarized according to the assessment methods used.

6.1. Pulsatile ocular blood flow findings

Reduced pulsatile OBF has been observed in patients with POAG and especially NTG (Silver et al., 1989; Schmidt et al., 1998; Fontana et al., 1998; Trew and Smith, 1991; Findl et al., 2000; Kerr et al., 1998). Furthermore, although the reductions were more pronounced in patients with visual field loss, they were seen before visual field defects were observed (Fontana et al., 1998). This is in contrast to other diseases, such as retinitis pigmentosa (Schmidt et al., 2001).

6.2. Angiography findings

Reduced blood flow in the retina, choroid and ONH in glaucoma patients has been demonstrated using angiography (Hitchings and Spaeth, 1977; Schwartz et al., 1977; Schwartz, 1994; Yamazaki et al., 1996; Tanaka, 1995; Arnold, 1995; Sugiyama et al., 2000). Both in the retina and choroid a delayed filing and especially a prolonged passage time have been described (Nasemann et al., 1994; Duijm et al., 1999). Whereas the reduction of retinal circulation occurs in HTG and NTG patients, the reduction in choroidal blood flow seems to be especially pronounced in NTG patients (Geijssen and Greve, 1995; Duijm et al., 1997). In the ONH three types of defects have been described: (a) local filling defects (Fig. 19), (b) slow filling, (c) increased leakage (Fig. 20). Filling defects and delayed filling have also been observed around the ONH, especially when using indocyanine green angiography (Nanba and Schwartz, 1983; O’Brart et al., 1997; Plange et al., 2001; Prünste, 1995).

6.3. Laser Doppler findings

6.3.1. Laser Doppler velocimetry

Results from LDV analyses in POAG patients and NTG patients show that OBF velocities were reduced. This reduction was associated with rheological alterations (Hamard et al., 1994).
6.3.2. Laser Doppler flowmetry
LDF has been used to describe differences between the OBF parameters in POAG and NTG patients and controls (Feke et al., 1995; Piltz-Seymour et al., 2001). As mentioned earlier, due to large inter-patient variations, the method is more suitable for intra-individual comparisons.

6.3.3. Heidelberg retina flowmeter
A number of studies using HRF have shown that OBF in the ONH and retina of glaucoma patients are reduced (Michelson et al., 1996; Michelson et al., 1998a, b; Harju and Vesti, 2001; Findl et al., 2000; Kerr et al., 1998; Hayashi et al., 2000; Ciancaglini et al., 2001), however this finding has not been uniform, with some studies showing no reduction (Hollo et al., 1996–97; Hollo, 1997). The reduction of OBF seems to be especially pronounced in the peripapillary area (Chung et al., 1999). Altitudinal visual field asymmetry is coupled with altered retinal circulation (Arend et al., 2000).

6.4. Laser speckle phenomenon findings
Results available from the literature to date have concentrated mainly on the validation of the technique and study treatment effects (Tamaki et al., 2001). In vivo studies confirm that laser speckling does provide a non-invasive technique for measuring blood flow in the retina and ONH, however it has not yet been used for assessment of OBF in glaucoma patients or comparison with normal controls.

6.5. Blue field entoptic findings
This technique has been used to study autoregulation (Riva et al., 1981). In glaucomatous eyes the response to IOP increase is different compared to normals (Grunwald et al., 1984). Leukocyte velocity correlates with visual function (Sponsel et al., 1990), a reduction can therefore be expected in glaucoma patients.

6.6. Retinal vessel analyzer
There are indications that glaucoma patients have on average relatively narrow arteries and dilated veins (Sugiyama et al., 2000). In addition local narrowing of the arteries at the ONH border has been described (Rader et al., 1994). However a systematic comparison between the size of the retinal vessels in glaucoma and non-glaucoma patients is not available in the literature yet.

6.7. Temperature
Corneal temperature correlates with retrobulbar hemodynamics, both in normals and glaucoma patients (Gugleta et al., 1999) and with finger temperature (Girardin et al., 1999). However, studies systematically comparing corneal temperature of glaucoma patients with normals have not yet been performed.

6.8. Color Doppler imaging findings
A number of CDI studies have found reduced peak systolic and diastolic velocities and increased resistivity indices in the retrobulbar vessels of glaucoma patients when compared with healthy normal controls (Fig. 21) (Galassi et al., 1992; Trible et al., 1994a; Michelson et al., 1995; Nicolela et al., 1996b; Nicolela et al., 1996c; Rankin et al., 1995; Rankin et al., 1996; Gherghel et al., 2000; Kaiser et al., 1997b; Cheng et al., 2001). Within individuals, retrobulbar blood flow reductions are greater in the eye with more damage (Nicolela et al., 1996a). In addition there are indications that the reduction precedes the damage (Costa et al., 1994). Patients that progress seem to have greater reductions and, within individuals, eyes with faster progression also have bigger OBF reduction (Kaiser et al., 1997b; Schumann et al., 2000).

6.9. Peripheral blood flow findings
Blood flow in the nailfold capillaries (Flammer, 1995) and in the micro-circulation of the skin (Drance et al., 1988; O’Brien and Butt, 1999) is reduced in glaucoma patients, especially after cold provocation. Furthermore blood flow velocity is also reduced in larger vessels (Nasemann et al., 1995). When peripheral blood flow in
glaucoma patients was compared with normal controls, baseline blood flow was on average slightly decreased, the difference however became very clear after cold provocation, especially in NTG patients (Fig. 22) (Flammer et al., 1987a; Drance et al., 1988; Gasser and Flammer, 1991).
6.10. Perimetry findings

Visual field defects often have a reversible component, which can be observed either by reducing IOP or pharmacological improvement of circulation, e.g. by the use of carbonic anhydrase inhibitors (CAIs) (Fig. 23) (Flammer and Drance, 1983a; Flammer and Drance, 1983b) or calcium channel blockers (Flammer et al., 1987a; Flammer and Guthauser, 1987; Gasser and Flammer, 1990; Gaspar et al., 1994). Theoretically, calcium channel blockers could improve the visual field by direct effects on the neural tissues. However the same improvement can be observed through breathing carbon dioxide (Pillunat et al., 1994), which indicated that it is therefore very likely that the short-term reversibility of visual field defects after calcium channel blocker intake is due to improved circulation (Fig. 24). Interestingly the improvement observed after the intake of CAIs is not correlated with IOP reduction (Flammer and Drance, 1983a). It is therefore reasonable to assume that a reversible damage due to vascular insufficiency can precede the irreversible damage in some cases.

7. Indirect signs of altered blood flow

There are a number of signs that point indirectly to the fact that at least in some glaucoma patients, blood flow is compromised. In the eye, changes in conjunctival capillaries (Unger and Jankovsky, 1967; Orgül and Flammer, 1995), local vasoconstriction in the retina (Rankin and Drance, 1996), increased prevalence of ONH hemorrhages (Drance et al., 2001; Begg et al., 1970; Kottler and Drance, 1976; Susanna et al., 1979; Drance et al., 1997; Siegner and Netland, 1996; Sugiyama et al., 1997; Orgül and Flammer, 1994), increased prevalence of venous thrombosis (Sonnsjo and Krakau, 1993; Malay et al., 1999; Klein et al., 2000) as well as gliosis like alterations (Fig. 25) have been described (Graf et al., 1993). In addition, evidence of increased prevalence of ischemic lesions in other organs has also been published, like: hearing problems (especially in patients with ONH hemorrhages) (Susanna and Basseto, 1992; Erb et al., 1996), silent myocardial ischemia (Fig. 26) (Waldmann et al., 1996; Kaiser et al., 1993a), and small ischemic lesions in the brain (Stroman et al., 1995; Ong et al., 1995; Corbett et al., 1985).
number of glaucoma patients have increased plasma levels of endothelin-1 (Sugiyama et al., 1995; Kaiser et al., 1995a,b; Flammer, 1997a; Cellini et al., 1997) and in the aqueous humor (Noske et al., 1997). However, the increase of circulating endothelin-1 is not specific to glaucoma and, for example, can also be observed in patients with autoimmune diseases (Flammer et al., 1984b; Haufschild et al., 2001) or in patients with Susac syndrome (Flammer et al., 2001a). GON-like damage could experimentally be induced by endothelin-1 application (Orgül et al., 1996a,b; Cioffi et al., 1995; Oku et al., 1999). Besides an increase of endothelin-1, there are other signs of compromised peripheral endothelial cell function in glaucoma (Henry et al., 1999). Endothelium dependent modulation is selectively reduced in subcutaneous resistance arteries isolated from patients with NTG (Buckley et al., 2002). Glaucoma patients also often have sleep disturbances (Marcus et al., 2001) and sleep apnea (Walsh and Montplaisir, 1982; Mojon et al., 1999a; Mojon et al., 1999b; Mojon et al., 2000; Onen et al., 2000). Finally clinical signs of vascular dysregulation are clearly increased, a phenomenon that will be discussed later in this review. A potential role of blood flow in glaucoma is further supported by the fact that treatment with calcium channel blockers seems in some patients to improve visual field prognosis as demonstrated in retrospective (Gasser and Flammer, 1990; Ishida et al., 1998; Netland et al., 1993) and prospective (Kitazawa et al., 1989; Koseki et al., 1999) studies.

8. Primary cause or secondary effect?

We have shown evidence to support the view that OBF is indeed reduced in at least a sub-group of glaucoma patients. Reduction was found in all ocular tissues tested so far, but especially in the choroid, the ONH and peripapillary area (Ulrich et al., 1993; Duijm et al., 1997; Wolf et al., 1993). The question arises whether this alteration of blood flow is just a consequence of the glaucomatous disease (either due to increased IOP or due to the GON) or whether there is a primary vascular component which potentially might be involved in GON pathogenesis, or even in aqueous humor dysregulation.

It goes without saying that (a) increased IOP can reduce OBF especially if autoregulation is malfunctioning, correspondingly IOP reduction improves OBF (Trible et al., 1994a,b); (b) in atrophic tissue, like GON, the number of blood vessels and therefore blood flow is reduced (Quigley et al., 1984). Does this alone explain the OBF reduction in glaucoma or should we assume that there is an additional, primary component?

The possibility that the OBF reduction is only a secondary effect alone is unlikely, for the following reasons: firstly, the blood flow reduction is not confined to the eye alone, but can also be found behind the eye and even in the periphery (Gasser and Flammer, 1991). Secondly, at least in some patients blood flow reduction precedes GON (Nicolela et al., 1996b). The disruption of OBF is also unlikely to be due to IOP alone as it is even more pronounced in NTG than HTG patients.
Likewise, optic disk hemorrhages occur at all stages of the disease and much more frequently in NTG than HTG patients (Drance et al., 2001; Healey et al., 1998). This indicates that the causes of disc hemorrhages cannot be explained by damage alone and even less by IOP. All these observations indicate that at least an important part of the OBF reduction is primary. Finally, experimental studies were able to produce glaucomatous-like optic nerve damage by application of endothelin-1 in the perineural region of the anterior ONH in animals with normal IOP (Orgül et al., 1996a, b).

9. Potential causes of OBF reduction

There is enough evidence to assume a primary (i.e. not caused by GON or IOP) component to the OBF reduction in glaucoma patients, and we must ask what this might be caused by. Theoretically there are three possibilities: (a) increased resistance to flow, (b) reduced perfusion pressure, (c) and increased blood viscosity. While there is little support in the literature to assume a change in blood viscosity, there are many indications for both increased resistivity to flow and decreased perfusion pressure. Increased resistivity could be caused by structural changes like anatomical variations, vasculitis or arteriosclerosis, or it might be due to a functional dysregulation of the vascular diameter. Reduced perfusion pressure might be due to an increased IOP or decreased blood pressure. We will first discuss the potential role of arteriosclerosis.

10. Arteriosclerosis

Arteriosclerosis occurs very frequently, especially in older subjects, and is the major cause of the most important cardiovascular events like myocardial infarction or cerebral vascular insults. Arteriosclerosis is also clearly associated with ocular diseases like central retinal arterial occlusions (Ahuja et al., 1999), anterior ischemic optic neuropathy (AION) (Lieberman et al., 1978; Bertram et al., 1994; Hayreh, 1999), venous occlusion (Pliszkiewicz et al., 1984) and possibly even with cataract (Street et al., 1996) and age-related maculopathy (Klein et al., 1997). Many text books also describe arteriosclerosis as a potential RF for GON.

It is not easy to assess and quantify arteriosclerosis in vivo. Nevertheless, there are indirect signs of arteriosclerosis such as increased fibrinogen (Mirshahi et al., 2001). There are also well known RFs such as smoking, systemic hypertension, diabetes mellitus or dyslipidemia. There are some indications that arteriosclerosis and its RFs might be weakly related to increased IOP (Wu and Leske, 1997; Klein and Klein, 1981; Bonomi et al., 2000). Experimental studies strongly support the hypothesis that ischaemia of the endothelial cells lining Schlemm’s canal may lead to an increased IOP (Nakabayashi, 2001). Recently a markedly elevated concentration of the endothelial leukocyte adhesion molecule-1 (ELAM-1) in the trabecular meshwork of glaucomatous eyes was described (Wang et al., 2001). ELAM-1 is a characteristic early marker of atherosclerotic plaques in the vasculature.

However, at a given IOP are patients with arteriosclerosis exposed to a higher risk of developing GON? Does arteriosclerosis increase the IOP sensitivity? Experimental studies indicate that arteriosclerosis may increase sensitivity to IOP (Hayreh et al., 1994a) and the expression “senile sclerotic glaucoma” was introduced in the literature (Geijssen and Greve, 1987; Geijssen, 1991). But, in contrast to what is normally assumed, GON is only weakly related to arteriosclerosis or its RFs (Flammer, 1997b). For example, while men tend to have more and earlier arteriosclerotic plaques than women (Stensland-Bugge et al., 2001), it is women who are at a higher risk of developing NTG (Orgül et al., 1995a). While obesity is a clear RF for both arteriosclerosis and for an increase in IOP (Mori et al., 2000), NTG patients tend to have a low body mass index (Leske et al., 1995; Gasser et al., 1999b). Hypercholesterolemia is a RF for increased IOP (Stewart et al., 1996) but not for NTG (Carter et al., 1990). Smoking is also associated with increased IOP (Kaimbo et al., 1997) but not with GON (Kaimbo et al., 2001). Although diabetes was initially considered a major RF (Becker, 1971), more recent studies were unable to confirm earlier observations (Quigley et al., 1994; Lepidi et al., 1997; Jonas and Grundler, 1998). While increased IOP is slightly associated with systemic hypertension, GON is clearly associated with systemic hypotension (Tielsch et al., 1995). All these observations indicate that, although OBF is reduced in glaucoma, arteriosclerosis may not be the main cause of this reduction and may not be a main RF for GON. Even carotid stenosis, which may have an impact on ocular perfusion is not, or very weakly associated with GON (Pillunat and Stodtmie- ter, 1988). Reduced life expectancy in such patients may however be a confounding factor. An interesting observation is that AION due to arteriosclerosis leads to a bland atrophy of the ONH, whereas an AION due to giant cell arteritis leads to excavation very much comparable to GON resulting from other RFs (Orgül et al., 1994; Danesh-Meyer et al., 2001). Giant cell arteritis is accompanied by a marked increase of circulating endothelin-1 (Pache et al., 2002a), which might induce a secondary vasospastic disorder in ocular perfusion (Flammer et al., 2001b). Why arteriosclerosis, although reducing ocular perfusion, may lead to ONH infarction but not GON will be discussed later.
11. Perfusion pressure

Perfusion pressure is defined as the difference between the arterial and venous pressure. In the eye, venous pressure is equal to or slightly higher than IOP. We have already discussed the role of increased IOP. We will now focus on blood pressure. While IOP is very weakly positively correlated with blood pressure (Bonomi et al., 2000), it is systemic hypotension which is clearly a RF for GON (Bonomi et al., 2000; Drance et al., 1973a; Demaillé et al., 1984; Kaiser et al., 1993a, b; Kaiser and Flammer, 1991; Hayreh et al., 1994b; Collignon et al., 1998; Bechetoille and Bresson-Dumont, 1994; Graham and Drance, 1999; Graham et al., 1995; Kashiwagi et al., 2001; Follmann et al., 1996; Freyler and Menapace, 1988; von Worch and Kaestner, 1985; Detry et al., 1996). This finding has been consistently reported for decades. It has been shown that among glaucoma patients who eventually lose their sight, the proportion of patients with systemic hypotension is clearly larger than in other glaucoma patients (Freyler and Menapace, 1988). It has also been demonstrated that blood pressure drops, when related to major events like hemorrhages, can lead to GON (Drance et al., 1973a, b). Furthermore, patients with orthostatic reactions have a higher chance of developing GON (Detry et al., 1994). Blood pressure on average is significantly lower, both in NTG patients as well as in HTG patients who progress despite normalized IOP (Fig. 27) (Kaiser et al., 1993a, b). Finally, both patients with an over-dip in blood pressure (Graham and Drance, 1999), or with no dip at all (Detry et al., 1996; Kashiwagi et al., 2001) at night, have a higher risk of developing GON. While there is no doubt that systemic hypotension increases the risk of GON, either alone or by increasing sensitivity to raised IOP, not all patients with low blood pressure develop GON.

Some patients with autonomic dysfunctions, due to Shy-Drager syndrome or autonomic diabetic polyneuropathy support very low blood pressures without suffering from GON (Flammer, 1994). How can we explain this? Under physiological conditions low perfusion pressure is compensated by low resistivity to flow (autoregulation). There are indications that this behavior might be altered in some subjects. It has been demonstrated that otherwise healthy subjects with vasospastic syndrome, do have altered blood flow regulation in their central retinal artery, as measured by CDI (Gherghel et al., 1999). This renders ocular perfusion directly dependent on perfusion pressure. Interestingly a very similar behavior is observed in patients with progression despite normal or normalized IOP (Gherghel et al., 2000), indicating that damage may occur if low perfusion pressure is combined with abnormal or insufficient autoregulation (Grunwald et al., 1984; Ulrich et al., 1988; Evans et al., 1999; Robert, 1989; Anderson, 1996). All these observations indicate that vascular regulation or dysregulation, respectively, might be key factors in the pathogenesis of GON. It is for this reason that vascular dysregulation will be discussed in more detail in the following chapters.

12. Vascular dysregulation

Blood flow through an organ is regulated by perfusion pressure and local resistance to flow. Local resistance in turn is controlled by the size of the local vessels. The regulation serves the purpose of ensuring an adequate supply of oxygen and nutrients reaching the tissues, as well as temperature and volume regulation. Many systems, like the autonomic nervous system, circulating hormones and endothelial cell layer, among others, are involved in this regulation.

Considering the complexity of this regulation, it is not surprising that in some conditions dysregulation may occur. Such a dysregulation implies an inadequate
arterial constriction (vasospasm) or, respectively, inadequate dilation when needed, often combined with widened arteries and especially veins in neighboring tissues (Flammer, 2001a). Therefore the term vascular dysregulation is more appropriate than vasospasm (Flammer et al., 1999). Nevertheless, vasospasms are responsible for inducing most of the symptoms, hence the historical use of vasospasm to describe the condition (Flammer et al., 2001b).

There are a number of pathological conditions that interfere locally with vessel size regulation, like diseased endothelial cells over an atherosclerotic plaque (Mombouli and Vanhoutte, 1999) or damage to autonomic nerve fibers (Miwa et al., 1998) for example. There are also however, conditions in which dysregulation occurs more globally, involving many different organs simultaneously or sequentially. These conditions are termed vasospastic syndrome (Meier et al., 1978) and can be grouped as primary or secondary (Flammer et al., 2001b).

Primary vasospastic syndrome is characterized by a tendency towards cold hands and sometimes feet, low blood pressure (Orgü et al., 1995b), a slim build, tendency to be meticulous, slower sleep onset (Pach et al., 2001) and a reduced dearth (Flammer, 2001a; Flammer et al., 2001b). It occurs more often in females than males. There is a genetic predisposition and the symptoms normally start between the ages of 10 and 20 years and mitigate as the patients age. In females it often, but not always, disappears or subsides after the menopause. Patients with migraines more frequently have a vasospastic syndrome (Gasser and Meienberg, 1991). However migraine and vasospastic syndrome are not identical and should not be confused (Lendvai et al., 1999; Nakamura et al., 2000). Although a patient’s history, e.g. cold hands, gives us some hints (Guthauser et al., 1988), vasospastic syndrome is usually diagnosed using nailfold capillary microscopy (Mahler et al., 1989). Its pathogenesis is not yet known. There are indications however that it is a consequence of a vascular endotheliopathy (Nakamura et al., 1999), with patients tending to have slightly increased levels of endothelin-1 circulating in the blood (Zuccarello, 2001).

Secondary vasospastic syndrome occurs as a consequence of other diseases, such as autoimmune diseases like multiple sclerosis, chronic polyarthritis, lupus erythematosus, some types of infections such as HIV, or other conditions like brain hemorrhages. In contrast to primary vasospastic syndrome, secondary vasospastic syndrome can manifest itself anytime during a patient’s life. It is associated with a marked increase of circulating endothelin-1. In these patients endothelin-1 is produced not only by the endothelial cell layer, but also by additional cells. The type of cells producing endothelin-1 depends on the underlying disease. For further reviews see Flammer et al. (2001b).

Raynaud’s disease is a special form of secondary vasospastic syndrome. It involves mostly, though not exclusively, the hands. Vasospastic syndrome, however, describes a more global condition which is similar, but not identical to Raynaud’s syndrome (Flammer et al., 2001b).

13. Vascular dysregulation and OBF

Involvement of ocular circulation in vasospastic syndrome was postulated in the early 1980s when it was noted that patients with a primary vasospastic syndrome often had diffuse or glaucomatous-like visual field defects which the patients were not aware (Gasser et al., 1986; Gasser and Flammer, 1987). In these patients the ONHs were mostly normal, or sometimes pale. It was assumed that the reduction of blood flow occurred mostly in the choroid (Flammer and Guthauser, 1987). The visual field defects in such patients fluctuate markedly and can spontaneously disappear. An improvement in visual field, in parallel with improvement of peripheral blood flow was observed after the uptake of calcium channel blockers (Fig. 28) (Flammer, 1993; Flammer and Guthauser, 1987). Conversely deterioration in visual field in parallel with deterioration of peripheral blood flow after cold stimulation has been described (Guthauser et al., 1988). These observations led to the hypothesis that the eye may sometimes be involved in vasospastic syndrome and led to the term ocular vasospastic syndrome (Flammer et al., 1992). With the advent of modern techniques to measure blood flow it was made possible to demonstrate that the eye may indeed be involved in the syndrome and that these patients often have altered OBF autoregulation (Fig. 29) (Gherghel et al., 1999; Evans et al., 1999) and that the choroidal circulation is indeed involved (Hasler et al., 2002).

Furthermore it has been shown that ocular vasospastic syndrome might be an RF for central arterial occlusions (Flammer et al., 2001b), retinal vein occlusions (Messerli and Flammer, 1996), AION (Kaiser et al., 1995a) in young patients and also central serous chorioretinopathy (Prünte and Flammer, 1996). We will now focus on the relationship between vascular dysregulation and glaucoma.

14. Vascular dysregulation and glaucoma

Due to the fact that primary vasospastic syndrome can involve the eye and can induce glaucomatous-like visual field defects, it was postulated that this syndrome might also be an RF for GON—especially in patients with NTG (Flammer et al., 1987a; Broadway and
Drance, 1998). Subsequent studies have indeed shown that glaucoma patients are more often vasospastic than controls (Gasser and Flammer, 1991; Rojanapongpun and Drance, 1993b; O’Brien, 1998).

The fact that we see improvements in circulation or visual field in some glaucoma patients after treatment with calcium channel blockers (Flammer and Guthausser, 1987; Guthauser et al., 1988) or CO₂ breathing (Pillunat et al., 1994; Harris et al., 1994) is indirect proof of a vascular dysregulation involved.

Vasospasms also seem to be associated with GON progression (Gherghel et al., 2000). Glaucoma patients often have increased endothelin-1 plasma levels (Flammer, 1997a). Patients with chronic glaucoma, especially those with NTG, suffer more often from migraine (Phelps and Corbett, 1985; Wang et al., 1997).

Why is there a potential relationship between vasospastic syndrome and GON (Flammer et al., 1999)? Vasospastic syndrome could interfere with OBF in two ways. Firstly, these patients tend on average to have lower blood pressure, thus they may have periods of low perfusion pressure. Secondly, glaucoma patients often have disturbed autoregulation (Sponsel et al., 1997), which might be a manifestation of the primary vasospastic syndrome (Gherghel et al., 2000). Reduced OBF might therefore in some cases be the result of an insufficient adaptation to low perfusion pressure (Fig. 30) (Gherghel et al., 2000).

Although ONH blood flow is reduced physiologically at night (Osusky et al., 2000) and retrobulbar blood flow in glaucoma patients with nocturnal over-dipping in systemic blood pressure is altered (Gherghel et al., 2001), a disturbed nocturnal hemodynamic has not yet been directly proven in glaucoma patients (Harris et al., 1997).

Vasospastic syndrome can be considered as an RF occurring independently of IOP, but acting in concert with IOP by rendering the eye more sensitive to IOP. Such a view is supported by the observation that GON occurrence and progression is in patients with vasospastic syndrome, more closely related to IOP than in...
non-vasospastic glaucoma patients (Schulzer et al., 1990).

As stated earlier, increased IOP is sufficient to induce glaucomatous damage. On the other hand damage can occur in patients who have never had increased IOP. At least some of these patients have some type of vascular insufficiency. Such chronic disturbance of ocular perfusion may in rare cases induce glaucomatous damage by itself. However, the observation that IOP reduction is beneficial, even in NTG patients (Collaborative Normal-Tension Glaucoma Study Group, 1998), indicates that GON results in the majority of cases from an interplay of different RFs.

Chronic dysregulation of blood flow might not only lead to GON but may also, in some cases, damage aqueous humor outflow (Nakabayashi, 2001). Little is known about such a potential relationship. Nevertheless NTG patients who subsequently develop increased IOP have been described (Schwartz et al., 1991). Therefore a dysregulation of aqueous humor dynamics and OBF may in some cases be related, but even if their occurrence is mutually independent, they may act synergistically.

15. Rheological factors

Theoretically not only low perfusion pressure or increased local resistance, but also systemic or local increases in blood viscosity can lead to reduced blood flow. Whereas some authors find slight changes (Liu et al., 1997; Wu and Li, 1993; Ge et al., 1993; Ge, 1992), this could not be demonstrated in all studies (Carter et al., 1990). However, this does not exclude local increase in blood viscosity. Decreased erythrocyte deformability (Ates et al., 1998), hyperaggregability of the erythrocytes (Hamard et al., 1994), and altered red blood cell membrane integrity (Carter et al., 1990) have been described in primary open angle glaucoma. In addition the coagulation cascade is activated at least in some glaucoma patients (O’Brien et al., 1997). Also increased aggregability of thrombocytes has been described (Hoyng et al., 1992; Bojic and Skare-Librenjak, 1998–99). Such changes in the constituents of the blood could be independent RFs occurring in some patients. It is also possible, however, that these rheological changes are a consequence of the vascular changes. The endothelial cells not only release vasoactive factors abuminally which influence the smooth muscle cells and the pericytes but also intraluminally to influence blood cells. The aggregability of thrombocytes is influenced by endothelial cells (Nishijima et al., 2001). Increased platelet adhesion occurs in the reperfusion phase (see later). An underlying endotheliopathy could therefore not only lead to vascular dysregulation but also to rheological alterations. Further studies are needed to clarify these potential interactions.

16. Ocular blood flow and glaucomatous optic neuropathy

The functional loss of vision in glaucoma is caused by cell death of retinal nerve cells and their axons. This is at least partially due to apoptosis (Garcia-Valenzuela et al., 1995; Murakami and Okisaka, 1998), a genetically predetermined program of cell death, which can be activated by many different factors depending on the situation. It is important to appreciate that apoptosis is a fundamental biological process and is part of the natural cell life cycle. Its inappropriate activation can, however, lead to disorders associated with pathological loss of cells. The exact mechanisms which induce apoptosis in glaucoma are not known, but we do know that apoptosis results in the destruction of the retinal ganglion cells, their axons and the supporting glial cells leading to a characteristic excavation of the ONH (Hernandez, 2000).

It is not yet known whether glial cells are damaged first, followed by neural cells, and indeed whether the axons are damaged before the ganglion cell body (Agapova et al., 2001). This review will not address all the different mechanisms discussed in the literature that may eventually lead to cell death and finally glaucomatous damage. We will focus only on the potential role of decreased blood flow.

It is known from both experimental animal studies and clinical studies in humans that ischemia can lead to ganglion cell death and ONH atrophy (Katai and Yoshimura, 1999; Cioffi and Sullivan, 1999). Reduction of OBF also results in glial fibrillary acidic protein expression in the retinal Muller cells (Osborne et al., 1991). In the ONH of glaucoma patients, astrocytes express not only major histocompatibility complex-I (MHC-I) but also MHC-II (Yuan and Neufeld, 2001). Interestingly hypoxia is able to induce MHC-II expression in ONH astrocytes in culture (Yang et al., 2001).

A number of clinical studies indicate that glaucoma patients do have an increased prevalence of autoimmune diseases (Cartwright et al., 1992; Wax, 2000). Autoimmunity may in some situations contribute to, while in other situations protect against, damage (Schwartz and Yoles, 2000). Besides the fact that some of the autoimmune responses may be secondary to the damage, we would like to stress that autoimmune diseases most often lead to an increase in endothelin-1 production, thereby inducing a secondary vasospastic syndrome (Flammer et al., 2001b). Autoimmune diseases therefore may change OBF and thereby may contribute to the development of GON.
Furthermore ONH astrocytes of glaucoma patients express nitric oxide synthase 2 (NOS 2) (Liu and Neufeld, 2000). Pharmacological inhibition of these enzymes in experimental animal models, partially inhibits the development of GON in rats with increased IOP (Neufeld et al., 1999). NOS 2 expression in glial cells can be induced in vitro by both mechanical (Liu and Neufeld, 2001) and hypoxic stress (Kobayashi et al., 2000). This observation stresses again the possibility that mechanical and vascular factors might lead to the same end result or that they might work synergistically.

We would like to emphasize however that glaucomatous atrophy is clearly different from the bland atrophy observed, e.g. after retinal arterial occlusion or after a non-arteritic AION. Hypothetically there are three ways to explain the difference:

(a) Ischemia may only lead to glaucomatous damage together with a damaging IOP. The occurrence of NTG makes such an explanation unlikely.

(b) A chronic ischemic lesion might lead to a different damage compared to an acute ischemic lesion. The fact that ocular ischemic syndrome, as seen in patients with occlusive carotid artery disease, does not necessarily lead to GON, makes this hypothesis unlikely (Costa et al., 1997; Pillunat and Stodtmeister, 1988).

(c) The damage may more likely be a consequence of reperfusion injury rather than pure ischemia (Flammer, 2000; Flammer, 2001b). This hypothesis explains most of the experimental and clinical observations and therefore will be discussed further.

17. The concept of reperfusion damage

It has been known for a long time that IOP fluctuations are more strongly correlated to progression of visual field damage than the level of mean IOP (Asrani et al., 2000; Flammer et al., 1982; Weber et al., 1993), with different therapies exerting different effects on this fluctuation (Orzalesi et al., 2000). Likewise, blood pressure fluctuations are more damaging than a steady decrease in blood pressure (Kashiwagi et al., 2001). Furthermore, as we have seen previously, while arteriosclerosis is only weakly related to GON, vascular dysregulation seems to be a major RF. In contrast to atherosclerosis, vasospastic syndrome interferes with OBF autoregulation, therefore a physiological normal or pathologically increased fluctuation of perfusion pressure leads to parallel OBF fluctuations. The observations highlighted in this review suggest fluctuating blood flow might be a stronger RF for GON than a stable decrease in blood flow. It was therefore suggested that the real mechanism of damage, causing GON, might be a repeated small reperfusion injury resulting from OBF fluctuation (Flammer, 2001b).

During reperfusion the production of free radicals (Schempp and Elstner, 1998), and thereby oxidative stress, is markedly increased. The mitochondria, present in especially high numbers in the axons of the ONH (Andrews et al., 1999), play a major role in reperfusion injury. An increase of free radicals also interferes with the re-uptake of glutamate (Vergun et al., 2001), leading to an increased extra-cellular glutamate concentration and excototoxicity (Bonne and Muller, 1998). During excototoxicity, the production of nitric oxide is also increased. The combination of increased nitric oxide production (in the astrocytes) combined with an increase of superoxide (mostly produced in the mitochondria of the axons) leads to the formation of the very damaging peroxynitrate (Haefliger et al., 1999a, b). Analysis of lymphocytes circulating in the blood revealed an over-expression of the P53 gene and a reduced expression of the Xeroderma pigmentosum gene (XP/GC) supporting the hypothesis that reperfusion injury is involved in the pathogenesis of GON (Golubnitschaja-Labudova et al., 2000). The fact that polymorphic glutathione S-transferase M1 is a risk factor for glaucoma supports the assumption that oxidative stress may play a role (Juronen et al., 2000). The production of free radicals might also be involved in the damage of the aqueous humor outflow system and may perhaps explain why radiologists more often have ocular hypertension (Scurti et al., 1992). We have already mentioned that ischemia/reperfusion induces similar changes as does mechanical stress. We would like to emphasize again that the mechanical and vascular theory do not exclude each other (Flammer, 1985b).

18. Conclusions and therapeutic consequences

Although it remains somewhat difficult to clinically measure OBF in the eye, techniques now exist which enable ophthalmologists to assess hemodynamic properties in and behind the eye at least for research purposes. All the different studies, using different types of instruments, point in the same general direction indicating that on average blood flow is decreased in some glaucoma patients, especially in NTG patients and in patients that progress despite normalized IOP. Furthermore this decrease in blood flow, although related to the extent of damage, can precede GON and is not confined to the eye alone. This would indicate that at least one component of the blood flow reduction in glaucoma is of primary nature. The exact cause of this is not known. However, current evidence suggests that a main factor is a general vascular dysregulation, called vasospastic syndrome, which leads to low blood
pressure and thus low perfusion pressure on the one hand, and on the other to disturbed autoregulation and an inability to adapt to increased IOP or decreased blood pressure. This results in unstable ocular perfusion. Cell death, partly through apoptosis, as well as a remodeling of the ONH is probably not just mediated by mechanical forces and ischemia, but also by reperfusion injury.

The accepted form of treatment for glaucoma remains IOP reduction. It not only reduces mechanical pressures but may also increase blood flow, at least in patients with disturbed autoregulation. Despite evidence of a vascular component in glaucoma for decades, additional treatment of blood flow in glaucoma is only just starting to be accepted as a possibility. The result is that new treatment options are now being investigated in clinical and laboratory settings, including improving ocular perfusion dynamics, influencing vascular dysregulation or protecting neural cells directly.

By virtue of the growing body of evidence supporting the role of ocular hemodynamics in glaucoma, it would be desirable to establish the influence of current and future glaucoma drugs on OBF. Besides the relatively well established knowledge based on in vitro and animal studies, relatively little information is available for human glaucoma patients. Results from in vitro or animal studies provide valuable information, however, the conclusions from these studies cannot automatically be applied to humans. In addition there are some indications that compared with healthy controls, patients with vascular dysregulation have a different sensitivity to vasoactive drugs.

Among the IOP lowering drugs, CAIs have a beneficial influence on ocular perfusion (Dallinger et al., 1998). Acetazolamide, e.g. produces a short-term improvement in visual field (Flammer and Drance, 1983b), and dorzolamide, a topical CAI, increases optic nerve oxygen tension (Stefánsson et al., 1999; Cour et al., 2000). There are also a number of non-IOP lowering drugs that might be of help. The literature on drug effects on ocular perfusion is huge and sometimes contradicting. We plan to summarize this in a subsequent review.

19. Future directions

In looking forward, we need to look at several aspects. The first is the technology we use to measure OBF. Although this has improved greatly, there is still much improvement needed. Using current techniques most measurements are of blood velocity. What is missing to a greater extent are direct data on blood flow in specific tissues of the eye.

Different sub-groups of glaucoma patients may behave in unique ways. More information about these sub-populations is needed.

Blood flow in an individual is not constant, but depends on the physiological and emotional conditions of the patient. In looking at glaucoma and OBF, we need to establish which data best reflect the glaucoma-tous patient as compared with normals.

Once we have all these parameters, we will be in a stronger position to evaluate both the impact of OBF in glaucoma and the relevance of targeting OBF in treatment modalities.

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