Choroidal Blood Flow and Progression of Age-Related Macular Degeneration in the Fellow Eye in Patients with Unilateral Choroidal Neovascularization

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PURPOSE. Cardiovascular risk factors such as smoking, hypertension, and atherosclerosis seem to play an important role in the development of choroidal neovascularization (CNV). Recent studies have also provided evidence suggesting that choroidal and retinal blood flow is decreased in patients with AMD. On the basis of these results, the hypothesis for this study was that lower choroidal blood flow is associated with an increased risk of CNV in patients with AMD.

METHODS. Forty-one patients with unilateral choroidal neovascular AMD were included in this observational longitudinal study. The fellow eyes of the patients served as study eyes. Subfoveal choroidal blood flow (FLOW) and fundus pulsation amplitude (FPA) were assessed with laser Doppler flowmetry and laser interferometry, respectively. A multivariate COX-regression model was used to test the hypothesis that choroidal perfusion parameters are associated with the development of CNV.

RESULTS. Of the 37 patients that were followed up until the end of the study, 17 developed CNV and 20 did not. The univariate COX-regression analysis shows that lower FLOW, systolic blood pressure, intraocular pressure, and FPA are risk factors for development of CNV. Moreover, the more advanced the AMD in the study eye, the higher the risk for CNV to develop in the fellow eye. Multivariate COX-regression analysis indicated that only FLOW (P = 0.0071), FPA (P = 0.0068), and staging (P = 0.051) had statistically significant influences on the progression to CNV.

CONCLUSIONS. The present study indicates that lower choroidal perfusion is a risk factor for the development of CNV in the fellow eye of patients with unilateral CNV. (Invest Ophthalmol Vis Sci. 2010;51:4220–4225) DOI:10.1167/iovs.09-4968

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The macula has the highest concentration of photoreceptors, which facilitate central vision and permit high-resolution visual acuity. Age-related macular degeneration (AMD) is the leading cause of severe and irreversible loss of vision in developed countries.1–4 Because of the changes in the population pyramid and the aging population in the Western world, this disease will gain even more importance over the next decades.

In recent years our understanding of the mechanisms underlying AMD has increased significantly. Most important, advances have been made in unveiling the associated genetic factors.3, 4 Other risk factors that have been identified are mainly of cardiovascular origin. A large number of studies have shown that hypertension and atherosclerosis are associated with AMD.5–8 In addition, the Blue Mountains Eye Study has suggested that focal arteriolar narrowing and arteriovenous nicking in the retinal vasculature are associated with the incidence of AMD.9 Based on epidemiologic data, an association between AMD and stroke has also been postulated,10,11 most likely due to generalized atherosclerosis. Further important risk factors include smoking12, 13 and age.14–16

Based on these results, it may be hypothesized that vascular factors play a role in the pathogenesis of the disease. Several cross-sectional studies have provided evidence suggesting that choroidal and retinal blood flow is decreased in AMD.14–18 The presence of abnormal choroidal circulation induces ischemia and hypoxia, which may trigger the development of angiogenesis. In late stages of AMD, angiogenesis leads to the formation of choroidal neovascularization (CNV), which can cause severe visual impairment by disrupting normal macular function.19 Vascular factors have also been implicated in the development of early AMD, including changes in the RPE and the formation of drusen.19 Cross-sectional studies have shown that it is difficult to evaluate whether reduced choroidal blood flow is a risk factor for the development of CNV or rather a consequence of the processes in the outer retina. In a longitudinal study, the development of CNV and visual loss were associated with lower choroidal circulatory parameters at baseline, indicating a role in the pathogenesis of CNV.20 In the present study, we tested the hypothesis that lower choroidal blood flow may be associated with an increased risk of development of CNV in the fellow eyes of AMD patients with unilateral CNV.

MATERIALS AND METHODS

Subjects

Forty-one patients with AMD were included in this trial. Approval from the Ethics Committee of the Medical University of Vienna was obtained, and the study was performed according to the tenets of the Declaration of Helsinki. The nature and possible consequences of the
study were explained, and all subjects gave written informed consent before participating. Each subject passed a screening examination that included a full ophthalmic examination. Only eyes with unilateral CNV secondary to AMD were included. In these, visual acuity had to be 20/40 or less, and a documented history of CNV was required. All participants had visual acuity of 20/32 or better and no advanced AMD in the fellow eye. The study eye was further staged according to the Clinical Age-Related Maculopathy Staging System.21 This grading was based on stereoscopic color photographs of the fellow eye. All eyes were categorized as either stage 2, characterized by approximately ≥10 small drusen (<0.5 mm in diameter) or <15 intermediate drusen (≥0.5 mm, but <1.25 mm in diameter) or pigment abnormalities associated with AMD, or stage 3, characterized by ≥15 intermediate drusen or any large drusen (≥1.25 mm in diameter). The absence of CNV in the study eye at baseline was confirmed by fluorescein angiography. Grading was based on fundus photographs by two independent investigators. In case of disagreement, a third investigator was involved, and discrepancies were resolved by open adjudication. Exclusion criteria were diabetes, glaucoma, and opacity of ocular media to a degree that affected the blood flow measurements.

Study Protocol
The duration of the study was 3 years. One screening visit and a maximum of seven study visits were scheduled for each subject. During the screening visit, inclusion and exclusion criteria were checked. On study days, measurements of blood pressure, pulse rate (PR), and ocular hemodynamic parameters (laser interferometry and laser Doppler flowmetry) and of intraocular pressure (IOP) were performed after a resting period of at least 20 minutes, to assure a stable hemodynamic condition. At all study visits, the same order of measurements was kept: blood pressure and PR, followed by laser interferometry, laser Doppler flowmetry, and finally applanation tonometry. Evaluations of laser interferometric data and laser flowmetry data were performed in a masked fashion. All measurements were performed twice a week. Accordingly, follow-up visits were scheduled every 6 months. A maximum of ±2 weeks’ deviation was allowed for each of these visits. Development of CNV in the study group was defined as the endpoint of the study (time to CNV). The influence of the following parameters on the development of CNV over time was analyzed: age, sex, eye (left or right), history of systemic hypertension (based on physician diagnosis, use of antihypertension drugs, or both), smoking status (current smoker, past smoker, or nonsmoker), staging of the study eye (according to the criteria), type of CNV in the fellow eye (classic, minimally classic, or occult), time from the development of CNV in the worse eye to inclusion in the study (time from CNV), subfoveal choroidal blood flow (FLOW, as assessed with laser Doppler flowmetry), fundus pulsation amplitude (FPA, as assessed with laser interferometry), systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), PR, and IOP.

Measurements
Blood Pressure and PR. SBP, DBP, and MAP were measured on the upper arm with an automated oscillometric device (HP-CMS patient monitor, Hewlett Packard, Palo Alto, CA). PR was automatically recorded with a finger pulse oximetry device (HP-CMS patient monitor; Hewlett Packard).

Intraocular Pressure. A slit lamp–mounted Goldmann applanation tonometer was used to measure IOP. Before each measurement, 1 drop of 0.4% benoxinate hydrochloride combined with 0.25% fluorescein sodium was used for local anesthesia of the cornea.

Laser Doppler Flowmetry. Subfoveal choroidal blood flow was measured by laser Doppler flowmetry in the nondilated study eye. In this technique, the vascularized tissue is illuminated by coherent laser light.22 Light scattered by the moving red blood cells undergoes a frequency shift. In contrast, static tissue scatterers do not change the light frequency, but lead to randomization of light directions impinging on red blood cells. Hence, red blood cells receive light from numerous random directions. As the frequency shift is dependent not only on the velocity of the moving red blood cell, but also on the angle between the wave vectors of the incident and the scattered light, scattering of the light in tissue broadens the Doppler shift power spectrum. From this spectrum, the following flow parameters are calculated: mean red blood cell velocity in hertz, blood volume, and FLOW in arbitrary units based on the theory of Bonner and Nossal.23 Briefly, velocity in hertz is the magnitude of Doppler shift induced by the moving particles, volume is the amount of Doppler-shifted light proportional to the number of red blood cells within the scattering volume, and FLOW is calculated as constant × velocity × volume.

In the present study, a compact laser Doppler flowmeter was used for the measurements of the choroidal blood flow parameters.24-25 The laser beam of a single mode laser diode (985 nm, 90 μW at the cornea) is delivered to the eye via a confocal optical system. The beam diameter at the fundus is nominally 12 μm. The light is collected by a bundle of six fibers with a core diameter of 110 μm, which are arranged on a circle with a diameter of 180 μm (indirect detection). All measurements were performed in the fovea by asking the subject to directly fixate the beam, which appeared as a small red dot. Measurements were analyzed using a NeXT Computer–based program (Redwood City, CA) described elsewhere.26 All readings lasted for at least 60 seconds, but not longer than 180 seconds. Portions in which the signal was disturbed by blinks or inadequate target fixation were removed from analysis. The latter was identified when the direct current portion of the reflected light was not any more within ±10% of the level during the start of measurement.27

Fundus Pulsation Amplitude. Synchronous pulsations of the ocular fundus were recorded by laser interferometry in the nondilated study eye.28 The method uses a single-mode laser beam with a wavelength of 780 nm for illumination of the subject’s eye. The power of the laser beam is approximately 80 μW at a beam diameter of 1 mm. The light is reflected at the anterior surface of the cornea and at the laser beam of a single mode laser diode (985 nm, 90 μW at the cornea) is delivered to the eye via a confocal optical system. The beam diameter at the fundus is nominally 12 μm. The light is collected by a bundle of six fibers with a core diameter of 110 μm, which are arranged on a circle with a diameter of 180 μm (indirect detection). All measurements were performed in the fovea by asking the subject to directly fixate the beam, which appeared as a small red dot. Measurements were analyzed using a NeXT Computer–based program (Redwood City, CA) described elsewhere.26 All readings lasted for at least 60 seconds, but not longer than 180 seconds. Portions in which the signal was disturbed by blinks or inadequate target fixation were removed from analysis. The latter was identified when the direct current portion of the reflected light was not any more within ±10% of the level during the start of measurement.27

Data Analysis
Univariate COX regression analysis was calculated to investigate the influence of baseline (age, time after CNV, time before CNV, sex, hypertony, smoking status, staging, type of CNV, and eye) and time-varying (FLOW, SBP, DBP, MAP, PR, IOP, and FPA) factors on the development of CNV over time. Risk factors with a univariate significant P-value were further analyzed in a multivariate COX regression analysis. Missing time-dependent variables were replaced by carrying the last value forward.

Analysis was performed with the public domain program R (ver. 2.4.0; server hosted by the Institute for Statistics and Mathematics, University of Vienna). P < 0.05 was considered statistically significant.

RESULTS
Twenty-three of the subjects were men and 18 were women. A total of 19 right eyes and 22 left eyes were included. Twenty-
six patients had systemic hypertension and 15 did not. Eighteen subjects were current smokers, 9 were past smokers, and 14 were nonsmokers. According to the Age-Related Maculopathy Staging System, 19 eyes were categorized as stage 2 and 22 eyes were categorized as stage 3. Of the worse eyes, 18 had classic CNV and 23 had minimally classic or occult CNV. Other patient characteristics are presented in the list of metric variables shown in Table 1. Four patients showed up at the baseline visit only; they were lost to follow-up and were not included in the analysis. Of the remaining 37 patients 17 developed CNV and 20 did not. Visual acuity at baseline did not correlate with either FLOW or FPA.

The univariate COX regression analysis shows that staging, FLOW, SBP, IOP, and FPA have statistically significant influence on the development of CNV in the study eye (Table 2). The higher the staging of the study eye the higher the risk of CNV. For all other significant variables (FLOW, SBP, IOP, and FPA) lower values were risk factors for CNV. The multivariate COX regression with the univariate significant variables indicates that FLOW, FPA, and staging have a statistically significant influence on the risk of developing CNV over time (Table 3). In accordance with the univariate analysis, the higher the staging and the lower the FLOW and FPA, the higher the risk of developing CNV over time. SBP and IOP did not show up as significant risk factors in the multivariate analysis anymore.

Individual data for FLOW and FPA are shown in Figures 1 and 2 respectively. Data are separated for those subjects who developed CNV and those who did not. For both choroidal hemodynamic parameters, baseline values were lower in the group that developed CNV (FLOW, P = 0.012; FPA, P = 0.006). Moreover, this group showed a decline of ocular hemodynamic values over time (FLOW, P = 0.027; FPA, P = 0.041). By contrast, the group that did not develop CNV showed no changes in either FLOW (P = 0.23) or FPA (P = 0.88) over time.

**DISCUSSION**

Abnormalities of choroidal blood flow and its regulation have been reported in a variety of previous studies. As early as 1966, Duke-Elder proposed that many cases of AMD are caused by changes in the choriocapillaris. A large number of studies have since then shown that several quantitative measures of choroidal perfusion are abnormal in AMD compared with healthy control eyes. One study also reported that choroidal blood flow regulation during changes in perfusion pressure is abnormal in patients with AMD. Based on these results and data that indicate a reduction in the cross-sectional area of the choriocapillaris in AMD it has been hypothesized by several authors that reduced choroidal perfusion may contribute to the pathogenesis of AMD. For such a conclusion, however, longitudinal data are necessary to ensure that the observed reduction in choroidal blood flow is not an epiphenomenon, but is causatively involved in the disease process.

In the only longitudinal study, a total of 193 study eyes of patients with AMD were followed up for 1 to 5 years. Laser Doppler flowmetry measurements of choroidal blood flow...
were performed annually in these patients. During the observation period, CNV developed in 28 eyes of 18 patients. The eyes in which CNV developed had higher perfusion pressures and lower FLOW values and were more likely to have large drusen at baseline.

Our study extends this previous report in several ways. Only patients with unilateral CNV were included in the present study. Given the high risk for CNV in the study eye in these patients, we ended up with an almost equal number of patients who developed CNV over 5 years and patients who did not. By contrast, the group of patients studied by Metelitsina et al.20 was more inhomogeneous, with wide inclusion criteria (typical ophthalmoscopic features of AMD and no signs of CNV). Accordingly, the proportion of eyes in which CNV developed in their study was much smaller than in the present trial (28/193 eyes).

In addition, our follow-up was standardized, allowing us not only to assess baseline risk factors, but also to calculate the influence of time-dependent changes in a multivariate COX regression model. By contrast, the follow-up time was highly variable in the group of patients studied by Metelitsina et al.,20 ranging between 1 year (101 eyes) and 5 years (27 eyes), with most having short observation periods only. In keeping with this previous study, we observed a continuous decline in choroidal hemodynamic parameters over time in patients in whom CNV developed. Whereas, Metelitsina et al.20 observed a small increase in choroidal hemodynamic parameters over time in those eyes in which CNV did not develop, we did not see this effect in the present study. This result may be related to the smaller number of patients who did not develop CNV in the present study.

Another advantage of our study is that two independent methods for the assessment of choroidal blood flow were used and showed consistent results. The method used is important, because laser Doppler flowmetry (LDF) as used by Metelitsina et al.20 and also in the present study does not measure blood flow in absolute units. The FLOW values obtained are in arbitrary units and are influenced not only by the perfusion level within the scattering volume, but also by the scattering properties of tissue.27,35,37,38 Hence, the fact that lower LDF FLOW values are associated with the development of CNV does not necessarily mean that it is caused by the reduced perfusion of tissue; it could also be due to morphologic changes leading to changes in tissue scattering. Moreover, absolute blood flow values as obtained with this system may depend on the degree of cataract, because of light-scattering and absorption in the lens. Neither in the present study nor in the study by Metelitsina et al.20 was this a staging of cataract done, because it is unclear how LDF data should be corrected with changes in lens opacification. A detailed discussion on the limitations, the validity, and the specificity of data obtained with this technique can be found in recent review articles.22,38

The LDF results obtained in the study of Metelitsina et al.20 and the present trial were measured with two different systems. Whereas they used a fundus camera–based approach,59 we used a portable confocal system in the present study.24,25 In the fundus camera–based system an optical fiber is placed in a conjugated plane of the retina and is located at the center of the illuminated site.59 In the portable system, the scattered light is collected by a bundle of six optical fibers and guided to an avalanche photo diode.24,25 These fibers are arranged on a circle with a diameter of 180 μm, which is imaged around the incident beam on the fovea, thus representing the indirect mode of a confocal arrangement. This arrangement has the advantage that the central, 0° reflection from the retinal tissue is not included in the analysis.24,25

In addition, we used laser interferometric measurement of fundus pulsation to gain insight into choroidal perfusion. This technique also does not measure choroidal perfusion in absolute units, but is rather an indirect method providing insight into the pulsatile component of choroidal blood flow.26,40,41 Measurements of FPA are, however, independent of scattering

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**Figure 1.** Time course of FLOW during the 36-month observation period. Left: patients with no CNV in the study eye; right: patients with CNV. The development of CNV was the end point, after which no further measurements were obtained. Individual data are presented.

**Figure 2.** Time course of FPA during the 36-month observation period. Left: patients with no CNV in the study eye; right: patients with CNV. The development of CNV was the end point, after which no measurements were obtained. Individual data are presented.
properties of tissue and cataract, since only the reflected light from the retina and the cornea is used for analysis of the interferences. Only in the presence of very severe lens opacification are no measurements possible, because the amount of light reflected from the retina is not intense enough to allow detection of interferences in the plane in front of the eye. In such cases, no fundus pulsations can be quantified, but the inability to obtain these measurements never occurred in the present study. Details on the validity and specificity of the technique can be found in other papers.\cite{ref1, ref2}

Results with both techniques, however, show consistency, indicating that the lower the choroidal perfusion the higher the risk for CNV. Since no gold standard methods are available for the measurement of choroidal blood flow,\cite{ref3, ref4} we chose techniques that show good enough reproducibility to monitor longitudinal changes.\cite{ref5} In this previous study,\cite{ref6} the coefficient of variation for PFA and FLOW measurements was 4.9% and 11.5%, respectively.

The incidence of neovascular AMD in the study eye of the present cohort was high, but comparable to that in other studies. In the Submacular Surgery Trials (SST) Research Group, 370 fellow eyes with unilateral AMD were studied over a period of 2 years. CNV developed in 95 of those eyes and foveal geographic atrophy in 15 eyes.\cite{ref7} These data indicate an incidence of 12.8% per year for development of CNV, which is only slightly lower than that in the present study (15.3%) per year. In our study, however, no patients developed foveal geographic atrophy, most likely because of the small sample size. As in previous studies the stage of AMD in the study had a significant influence on the risk for AMD.\cite{ref8, ref9, ref10}

In conclusion, the present study indicates that lower choroidal perfusion is a risk factor for CNV in the fellow eye of patients with unilateral CNV.

References


