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CLINICAL INVESTIGATION

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Topical fundus pulsation measurements in age-related macular degeneration

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Abstract ● Background: The purpose of the present study was to investigate regional fundus pulsations in age-related macular degeneration (AMD) patients with subretinal neovascular membranes. ● Methods: Local fundus pulsation amplitudes (FPAs) were measured in 12 patients with AMD with classic neovascular membranes. Measurements were performed directly on the membrane and adjacent to the membrane. FPAs were assessed with a recently developed laser interferometric method. FPA measurements were performed in 12 healthy subjects at similar posterior pole locations. ● Results: In AMD patients FPAs were consistently lower when measured directly on the neovascular membrane ("inside") than at measurement sites around the membrane ("outside"). The difference in FPA was 26 ± 3% (mean ± SEM, range 13–40%, $P < 0.0001$). In healthy subjects, however, FPAs were significantly higher at the measurement points corresponding to "inside" points (15 ± 4%, $P < 0.0006$). ● Conclusions: We have shown that FPAs are reduced at classic neovascular membranes in patients with AMD. The mechanism behind this finding remains unclear. Hence, future studies have to ascertain whether this observation is associated with changes in fundus layers or with local choroidal perfusion abnormalities.

Introduction

Although age-related macular degeneration (AMD) is the most common cause of blindness in Western countries, little is known about the pathogenesis and pathophysiology of the disease [4]. To date, laser treatment is effective only in patients with choroidal neovascular membranes with well-demarcated boundaries exhibited during fluorescein angiography [11, 12]. Even in successfully treated cases, the development of central visual impairment is most often only postponed.

Recent studies indicate that choroidal blood flow is impaired in patients with AMD. Prolonged choroidal filling phase in fluorescein angiography has been assumed to indicate thickening of Bruch's membrane [13, 14]. Using colour Doppler imaging, increased pulsatility indices have been observed in ocular vessels, which suggests increased choroidal vascular resistance [6]. New methods with high topographic resolution are needed to more readily define the changes in ocular blood flow during AMD. The aim of this pilot study was to investigate regional differences in pulsatile blood flow in AMD patients with choroidal neovascularisations. Fundus pulsations were measured with a laser interferometric method which offers a high topographic resolution [18, 20]. Measurements were assessed at preselected points of the fundus based on fundus photographs and fluorescein angiograms.

Methods

The study protocol was approved by the Ethics Committee of Vienna University School of Medicine. Twelve patients with AMD (age range 69–88 years, mean 80.1) and a classical subretinal neovascular membrane gave written informed consent and were studied. Patients were included in the study based on the following criteria: fluorescein angiographic evidence of a classic choroidal neovascular membrane, no previous laser treatment in the study eye, visual acuity not less than 0.1 in both eyes. Exclusion criteria were evidence of any other retinal, choroidal or optic nerve vascular disease or limited view of the fundus due to cataract or vitreous haemorrhage. Additionally, 12 healthy subjects (age range 64–88 years, mean 72.8) were included in the study as controls. Exclusion criteria for this study group were evidence of any retinal, choroidal or optic nerve disease, visual acuity less than 0.8, diabetes mellitus and systemic hypertension (defined as systolic blood pressure, SBP, >165 mmHg; diastolic blood pressure, DBP, >95 mmHg).

Pulse synchronous pulsations of the ocular fundus were recorded with a laser interferometric method [18]. The method uses a high-coherence 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, which is below the limit set by the American National Standards Institute [1]. The light is reflected at the anterior surface of the cornea and at the fundus. The light from the front side of the cornea serves as a reference wave. This permits assessment of the relative distance changes between cornea and retina during the cardiac cycle from the interferences produced by the two reflected waves. These distance changes are in the order of several micrometres and are caused by the rhythmic filling of ocular vessels during systole and diastole. Hence, the distance between cornea and retina decreases during systole and increases during diastole [18]. The maximum distance change between the cornea and fundus during the cardiac cycle is called fundus pulsation amplitude (FPA) and is a measure of the pulsatile component of ocular blood flow [20].

The interferometer is coupled to a fundus camera, which allows real-time inspection of the retinal measurement point [18]. As the measurement point at the retina has a diameter of about 20–50 μm, high topographic resolution is achieved. Fundus pulsation measurements were performed at preselected points, on the basis of corresponding fundus photos and fluorescein angiograms not older than 1 week. Measurements were performed at a minimum of five points on the neovascular membrane and at a minimum of five points adjacent to the membrane. The retinal points were selected on two concentric circles, one on the membrane, the other adjacent to the membrane. When fundus pulsations were measured at sites directly on the membrane it was not always possible to record technically adequate interferograms. Measurements were included for analysis only when fundus pulsations could be recorded from at least three pulse periods. Otherwise, another point on the retina on the same circle was taken for measurement. Fixation was achieved by providing a fixation light to the contralateral eye. In the healthy subjects, fundus pulsation measurements were performed at similar posterior pole locations

A slit-lamp-mounted Goldmann Applanation tonometer (Nikon 105, Tokyo, Japan) was used to measure intraocular pressure (IOP). Before each measurement one drop of 0.4% benoxinate hydrochloride combined with 0.25% fluorescein sodium was used for local anaesthesia of the cornea. SBP and DBP were measured using an automated oscillometric device (HP CMS-patient monitor, Hewlett Packard, Palo Alto, Calif., USA). Mean arterial pressure (MAP) was calculated as 2/3 DBP + 1/3 SBP. Pulse rate (PR) was registered automatically from a finger pulse-oximetric device (HP CMS-patient monitor).

For data analyses the mean of the FPAs measured directly on the membrane (FPA_{in}) and the mean of the FPAs measured adjacent to the membrane (FPA_{out}) were calculated. Comparison between the two means was done by paired *t*-tests. Additionally, FPAs were compared between AMD patients and healthy subjects by means of an unpaired *t*-test. Data are presented as means ± SEM. The level of significance was set at $P < 0.05$.

Results

Characteristics of AMD patients as well as the percentage difference in FPA between measurements adjacent to the membrane and measurements directly on the membrane are summarised in Table 1. MAP was 106 ± 6 mmHg in the AMD group and 98 ± 5 mmHg in the healthy control group (n.s.). PR was not different between study groups (AMD group 72 ± 6 min^{-1}, control group 71 ± 4 min^{-1}). IOP tended to be higher in the AMD group (18.4 ± 2.1 mmHg) than in the control group (16.6 ± 1.7 mmHg; n.s.). Adequate measurements could be obtained for all AMD patients and all control subjects.

Table 1 Patient characteristics and percent difference in FPA from measurements adjacent to the membrane (100%) as compared to measurements directly on the membrane

Patient	Age	Sex	Visual acuity	Location	Percent difference in FPA
1	84	F	0.3	Juxtafoveal	15
2	83	F	0.5	Subfoveal	31
3	83	M	0.3	Juxtafoveal	24
4	88	F	0.1	Juxtafoveal	23
5	77	F	0.1	Juxtafoveal	25
6	86	F	0.1	Subfoveal	22
7	85	F	0.3	Juxtafoveal	18
8	81	M	0.4	Subfoveal	35
9	78	M	0.3	Juxtafoveal	13
10	69	M	0.4	Juxtafoveal	33
11	74	F	0.2	Subfoveal	32
12	73	M	0.5	Juxtafoveal	40

Table 2 Fundus pulsation amplitudes in μm (mean ± SEM) as measured directly on the membrane (FPA_{in}) and adjacent to the membrane (FPA_{out}). Measurements in age-matched individuals are taken at similar posterior pole locations

Patient	AMD patients		Healthy controls	
	FPA_{in}	FPA_{out}	FPA_{in}	FPA_{out}
1	4.0±0.2	4.7±0.5	3.3±0.3	2.8±0.3
2	2.2±0.3	3.2±0.3	2.9±0.3	2.3±0.3
3	2.2±0.3	2.9±0.3	6.0±0.6	4.6±0.5
4	2.0±0.2	2.3±0.2	4.6±0.8	4.3±0.4
5	2.8±0.4	3.5±0.4	3.6±0.5	3.5±0.3
6	3.2±0.3	3.9±0.2	2.6±0.2	2.5±0.2
7	3.4±0.2	4.0±0.3	4.0±0.3	3.6±0.3
8	4.0±0.5	5.4±0.4	5.4±0.5	4.4±0.5
9	3.8±0.3	4.3±0.4	2.8±0.3	2.7±0.3
10	4.2±0.5	5.6±0.3	4.2±0.4	3.6±0.6
11	3.7±0.3	4.9±0.2	3.3±0.3	2.7±0.4
12	2.5±0.3	3.8±0.3	3.8±0.4	3.0±0.6
All subjects	3.2±0.3	4.1±0.3	3.9±0.3	3.3±0.3

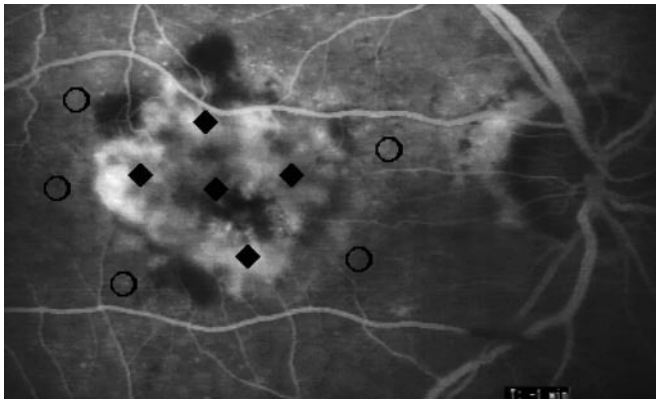

Fig. 1 Measurement points in a patient with a well-defined neovascular membrane (patient 2). *Black squares* Measurement points on the membrane, *open circles* measurement points adjacent to the membrane

In the AMD study group, FPA_{in} (3.2 ± 0.3 µm, 95% CI 2.7–3.7 µm) was significantly lower than FPA_{out} (4.1 ± 0.3 µm, 95% CI 3.4–4.7 µm, $P < 0.0001$; Table 2). The difference in FPA was 26 ± 3% (range 13–40%). In healthy subjects, however, FPAs were significantly higher at the measurement points corresponding to "inside" points (FPA_{in}: 3.9 ± 0.3 µm, 95% CI 3.2–4.5 µm; FPA_{out}: 3.3 ± 0.3 µm, 95% CI 2.8–3.8 µm; $P < 0.0006$). The difference in FPA was 15 ± 4%. However, differences in FPA_{in} and FPA_{out} between the two groups did not reach the level of significance.

Figure 1 shows a representative fundus photo from a patient with a classic neovascular membrane (patient 2). The fundus pulsations measured directly on the membrane were consistently lower.

Discussion

The current treatment of choroidal neovascularisations in AMD is restricted to precisely localised membranes [11, 12]; however, it has been shown that most patients have rather ill-defined choroidal neovascularisations [2]. Indocyanine green video angiography is useful for demarcation of neovascular membranes in some eyes, especially when the membrane is poorly defined by fluorescein angiography [3, 15, 16]. Preliminary results indicate successful laser treatments in these cases [16, 17]. Nevertheless, precise localisation of neovascular membranes is still impossible in a large number of patients. Moreover, the pathophysiologic mechanisms behind the development of neovascular membranes are still unclear. Hence, there is need for new methods to investigate ocular blood flow in AMD patients. For the investigation of local blood flow abnormalities, high topographic resolution is required.

Our preliminary results indicate that laser interferometric measurement of fundus pulsations might be of interest, although only the pulsatile component of choroidal blood flow can be assessed. In all AMD eyes under study, FPAs measured directly on the membrane were lower than at measurement sites adjacent to the membrane. In contrast, fundus pulsation measurements in healthy subjects were higher when the same posterior pole locations were compared. This means that in the healthy control group FPAs were higher in the macular region than in peripheral parts of the fundus, which is in keeping with our previous results in young healthy subjects [18]. Hence our results indicate that FPA measurements may be useful for investigation of local perfusion abnormalities associated with neovascular membranes, but additional studies in a larger population are needed to confirm this assumption.

The mechanism underlying the reduced FPA at the membrane in patients with AMD is not yet clear. In healthy subjects the fundus layer from which the main part of the light (at 800 nm) is reflected is most likely Bruch's membrane [5]. Hence the interference fringes, which are used for fundus pulsation measurements, are formed by the reflected waves from the front side of the tear film and Bruch's membrane [21], and FPA is therefore exclusively influenced by the choroidal circulation, with the exception of measurements in the optic disc [18, 19, 22]. When measurements are performed in AMD patients directly at the neovascularisation, the marked histological changes in the fundus layers must be taken into account [7]. For instance, breaks in Bruch's membrane, which are most likely caused by endothelial cells of neovascular membranes [9, 10], could strongly influence the ratio of re-emitted light from different fundus layers. Deviations from normals in fundus layer reflectivity have already been observed in patients with macular oedema [8].

Our results may reflect a truly reduced pulsatile blood flow in the choroidal circulation or may be attributed to changes in layer structure. Furthermore, a reduced fundus pulsation amplitude does not necessarily reflect reduced regional blood flow, as the ratio of pulsatile to non-pulsatile blood flow is too unpredictable. It might well be that the new choroidal vessels decrease local vascular resistance, leading to an increased but less pulsatile blood flow. Additional studies in AMD patients might clarify the mechanism behind reduced local fundus pulsations. Fundus pulsations should be investigated in areas of scarring, haemorrhage, retinal pigment epithelium detachment and extensive drusen. An investigation of the effect of laser treatment on topical fundus pulsations may provide information to show whether our results are associated with local perfusion abnormalities or changes in fundus layers.

Due to the small number of patients investigated in this study and the substantial interindividual variability of fundus pulsation measurements [20] we did not observe significant differences between the two study groups, al-

though we observed differences in FPA in the order of 20% between AMD patients and healthy control subjects. A sample size calculation [23] shows that 16 AMD patients and 16 control subjects would be necessary to detect a 20% difference between the two study groups (alpha level = 0.05, beta level = 0.2)

In conclusion, we have shown that FPAs are reduced at classic neovascular membranes in patients with AMD. The mechanism behind this reduction remains unclear. Hence future studies have to establish whether this observation is associated with changes in fundus layers or with local choroidal perfusion abnormalities.

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