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LASER INTERFEROMETRIC INVESTIGATIONS OF PULSATILE CHOROIDAL BLOOD FLOW: REVIEW AND NEW RESULTS ON THE VALIDITY OF THE TECHNIQUE

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ABSTRACT

A short overview of currently available ocular blood flow techniques is given. We have recently introduced a laser interferometric technique for the measurement of ocular fundus pulsation. The eye is illuminated by a single mode laser beam which is reflected at the anterior corneal surface and the fundus. The two re-emitted waves produce interference fringes from which distance changes between cornea and retina during the cardiac cycle can be calculated. These rhythmic changes in corneo-retinal distance are caused by the arterial pulsatile inflow of blood, which increases the ocular volume. The fundus pulsation amplitude (FPA) is the maximum distance change between cornea and retina during the cardiac cycle and is taken as a relative measure of pulsatile choroidal blood flow. The high reproducibility and the high sensitivity of the method are discussed. In addition, the present article reviews comparative measurement with other techniques for the assessment of choroidal blood flow, which validates the method. Furthermore, we present new data on a comparison of color Doppler imaging in the posterior ciliary arteries and laser interferometric measurement of FPA. Applications of laser interferometric measurement of FPA to study the physiology, the pharmacology, and the pathophysiology of the choroidal circulation are reviewed. In conclusion, FPA can be taken as a relative measure of pulsatile choroidal blood flow. The technique is particularly suitable for pharmacodynamic studies. [S1083-3668(98)01803-6]

Keywords fundus pulsation; laser interferometry; ocular blood flow; choroidal blood flow.

1 OCULAR BLOOD FLOW MEASUREMENTS—OVERVIEW

A variety of common ocular diseases, including glaucoma, age related macular degeneration, and diabetic retinopathy, are associated with ocular perfusion abnormalities. Hence there is considerable interest in the development of techniques for the specific investigation of retinal, optic nerve, and choroidal blood flow in man.

In experimental animals the microsphere techniques has been intensively used to study the regulation of ocular blood flow.[1–3] In addition, direct cannulation of ocular veins[4,5] and different clearance techniques[6–8] have been used for investigation of ocular blood flow in animals. Leukocyte dynamics have been successfully studied with a fluorescent nuclear dye of aricidine orange.[9]

In humans the investigation of ocular blood flow is obviously limited to noninvasive methods. For the retinal circulation a variety of methods have been proposed and several of them have been successfully used to investigate physiology, pathophysiology, and pharmacology of this vascular bed. Laser Doppler velocimetry can be used to study blood flow velocities in large retinal vessels.[10–12] By measuring the vessel diameter from fundus photos, the total volumetric blood flow can be calculated. The Doppler shift of laser light has also been used to study retinal microcirculation with scanning laser Doppler flowmetry.[13] The Doppler shift of ultrasound is the basis for the measurement of blood flow velocities in the central retinal artery with color Doppler imaging.[14,15]

Leukocyte velocity in the macular region has been measured using the blue field entoptic technique.[16,17] The blue field entoptic phenomenon can be seen best by looking into a blue light with a narrow optical spectrum. Under these conditions the motion of leukocytes in the macular region can be seen. Leukocyte velocity was also measured by means of the laser speckle technique, using the first-order spatial statistics of time-integrated speckle patterns.[18] Velocity of erythrocyte aggre-

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gates has been determined with fluorescein angiography using scanning laser technology in the para- and perifoveal capillary network.[19–21]

For the investigation of blood flow in the optic nerve only very few methods are currently available. Fluorescein angiography has been intensively used to study optic nerve head circulation,[22] but quantitative extraction of hemodynamic parameters is still difficult. By contrast, laser Doppler flowmetry measures a relative flux in the illuminated tissue,[23] although the depth from which the signal arises has not yet been determined.

The assessment of choroidal blood flow is difficult, because of the location of the vessels. Color Doppler imaging can be used to measure blood flow velocities in the posterior ciliary arteries (PCAs), which supply the choroidal vessels,[14] although the number of vessels under study cannot be determined due to the limited resolution of the method. Relative blood flow in the choriocapillaris can be assessed with laser Doppler flowmetry.[24,25] However, these measurements are limited to the macula, because the retina lacks vasculature in this region. Attempts have also been made to extract choriocapillaries blood flow from indocyanine and fluorescein angiograms.[26,27]

It has already been recognized for a long time that the intraocular pressure slightly varies during the cardiac cycle. Bynke and Schele[28] have shown that this ocular pressure pulse is mainly caused by the choroidal circulation. Finally, a procedure has been derived by which pulsatile ocular blood flow (POBF) can be calculated from the change of IOP over time.[29–32] For this purpose ocular pressure variations are converted into ocular volume changes based on the pressure-volume relationship proposed by Friedenwald.[33] This estimation of the pulsatile component of ocular blood flow is mainly based on two assumptions: First, POBF is not corrected for ocular rigidity of individual subjects, because this parameter cannot yet be assessed *in vivo*. Second, the venous outflow from the eye is assumed nonpulsatile.

More recently a laser interferometric method for the measurement of ocular fundus pulsation has been developed in our laboratory.[34] This technique was originally proposed by Fercher[35] and measures distance changes between the cornea and the retina during the cardiac cycle. As this laser interferometric method assesses a point measure of the ocular volume change caused by the rhythmic filling of the blood vessels it may be a more direct measure of POBF than ocular pressure pulse. As mentioned above the ocular volume increases when the arterial inflow through the arteries exceeds the venous outflow. This choroidal swelling during the systole leads to an increase in ocular blood flow and interocular pressure (IOP)[31,32] and to a reduction in the distance between cornea and retina. In other words the inflow of blood into the choroid during the systole leads to a slight reduction of the eye length. The aim of the present article is to review the work done so far with this technique, to discuss the advantages and the limitations of the technique, and to emphasize possible future directions of research. In addition, some new results supporting the validity of the technique are given.

2 MEASUREMENT PRINCIPLE

The method is described in detail by Schmetterer et al.[34] and is therefore only briefly described in the present article. The eye is illuminated by the parallel beam of a single mode laser diode with a wavelength (λ) of 783 nm. This light has a high temporal and spatial coherence. Hence the light reflected at both the front side of the cornea and the fundus can interfere. The wave reflected at the front side of the cornea is spherical, whereas the wave reflected from the retina is plane. Hence the two waves produce nonlocalized circular interference fringes. The path difference between the two re-emitted waves is twice the optical length of the eye. This principle has been used to measure the axial eye length by using an external interferometer and short coherence light.[36] Using high coherence light every distance change between cornea and retina leads to a corresponding variation of the interference order $[\Delta N(t)]$. This change in interference order can be evaluated by simply counting the fringes moving inwards and outwards through a fixed point. Changes in optical distance $[\Delta L(t)]$, corresponding to the cornea-retina distance changes, can then be calculated by $\Delta L(t) = \Delta N(t)\lambda/2$.

These changes in the corneo-retinal distance are caused by the periodic filling of the ocular vessels during the cardiac cycle. The maximum distance change between cornea and retina is called fundus pulsation amplitude (FPA). FPA in the macula is in the order of 4 μm in young healthy subjects. In the optic disc FPA is much larger than in the macula with values up to 10 μm, whereas in the peripheral retina FPA is normally slightly lower than in the macula.[34]

The beam focus at the fundus has a diameter of approximately 50–100 μm. The reflection from the posterior segment most likely occurs from the retinal pigment epithelium or Bruch's membrane. This assumption is supported by the observation that in low coherence interferometry the main reflections result from these fundus layers.[37–39] The corneal layer from which the light is reflected has not yet been definitely identified. Corneal thickness measurements[40,41] indicate that the main reflection occurs at the front side of the tear film, which is in keeping with theoretical considerations of the high difference in refractive indices of air and tear film. Hence fundus pulsation measurement only assesses choroidal blood flow, with the exception of measurements in the optic disc, which will be discussed later in this article. Longitudinal movements of the

eye as caused by head movements or by pulsations in the retrobulbar vasculature do not influence ocular fundus pulsation, because only relative changes between cornea and retina are measured with this method.

The interferences produced from the two reemitted waves are detected with a linear charge-coupled device array, which is placed in the center of the interference fringes. Each readout of the 256 pixels is plotted along the time axis, resulting in an artificial interferogram, which is a spatio-temporal display of ocular fundus pulsation. The distance change between cornea and retina can be evaluated by simply counting the fringes moving inwards and outwards during the cardiac cycle. An automatic fringe counting system cannot be used for the evaluation, because of lateral eye movements and blinks. In addition, the reflection from the retina shows a speckled structure,[17] which leads to local irregularities in the interference pattern.

The system is coupled to a fundus camera, which allows real-time inspection of the fundus during the measurement. Fundus pulsations can therefore be assessed at preselected fundus locations. However, measurements are restricted to an area of approximately 25° around the macula, because otherwise the two reflected beams cannot be superimposed.

It is obvious that not all pulse periods of the interferogram can be evaluated for the determination of FPA. This is the case if microsaccades, blinks, or tear-film cracks appear during the cardiac cycle. Hence the quality of measurement also depends on the compliance of the patient during measurement.

3 REPRODUCIBILITY AND VALIDITY

The reproducibility of laser interferometric measurement of fundus pulsation is excellent. As compared to other methods, such as color Doppler imaging,[42–44] pneumotonometric measurement of POBF,[44,45] and scanning laser Doppler flowmetry[46,47] the short-term intraindividual variability of fundus pulsation measurement is considerably smaller.

It is difficult to test the validity of FPA measurements because no gold standard for the measurement of choroidal blood flow exists. Some of the limitations of currently available methods have recently been summarized by Kiel.[48] Hence novel approaches can only be validated based on comparison between methods and investigations on the sensitivity to detect changes in choroidal blood flow. We have previously shown that there is a dose-dependent increase in FPA during infusion of isoproterenol, a β-adrenoceptor agonist.[49,50] Isoproterenol strongly increases cardiac output and pulse pressure amplitude (PPA) due to its positive inotropic effect. Hence, the observed increase in FPA is mainly caused by the increase in PPA, which is the driving force of pulsatile blood flow.[51] In addition, the high, dose-dependent reactivity of FPA to changes in arterial carbon dioxide tension (pCO_2) and the low reactivity to changes in arterial oxygen tension (pO_2)[52,53] validates the method. A strong dependence of the choroidal vasculature on pCO_2 and a low dependence on pO_2 has been observed previously in animal and human experiments.[2,6,24,25]

The situation is more complex when fundus pulsations are measured in the optic disc. The absolute fundus pulsation amplitude at the cup and at the neuroretinal rim is much higher than in other parts of the fundus.[34,54] This is presumably caused by the specific elastic properties of the eye coats in this region. Interestingly FPA shows a higher reactivity to pO_2 in the optic disc than in the macula,[51,52] which indicates that both the choroidal and the retinal circulation contribute to the signal.

Comparative investigations on POBF have so far been performed using laser interferometry and pneumotonometry[44,54,55] and laser interferometry and color Doppler imaging.[44] At baseline there is a strong association between FPA and pneumotonometrically determined ocular pressure pulse amplitude and POBF.[54] In addition, there is an association between FPA and the pulsatile blood flow velocity component in the PCAs.[44] A direct calculation of POBF from laser interferometric measurement of fundus pulsation is hampered by several reasons. Local FPA is only a point measure and the optical assessment of fundus pulsation is limited to an area of approximately 25° as mentioned above. Hence additional knowledge on the eye shape and local ocular rigidity would be required to estimate total POBF from our laser interferometric measurement.

In the present article we present additional results on the association between laser interferometric measurement of FPA and color Doppler imaging of blood flow velocities in the PCAs. FPA is a point measure of the ocular volume change during the cardiac cycle $[\Delta V(t)]$ assuming an infinitely small laser spot on the fundus. The ocular volume increases when the pulsatile inflow of blood through the arteries exceeds the nonpulsatile outflow through the veins. Color Doppler imaging, however, measures the time course of blood flow velocities in the PCAs $[v(t)]$. Blood flow through the PCAs is then give by multiplication of $v(t)$ with the cross sectional area of the vessels (Q), which cannot be determined *in vivo*. However, the transported blood volume into the choroid during one cardiac cycle is proportional to the area under the velocity curve assuming a constant vessel diameter in the PCAs.[56] Hence the change in choroidal volume is given by

$$\Delta V(t) = Q \int v(t)\, dt - BF_{\text{vein}},$$

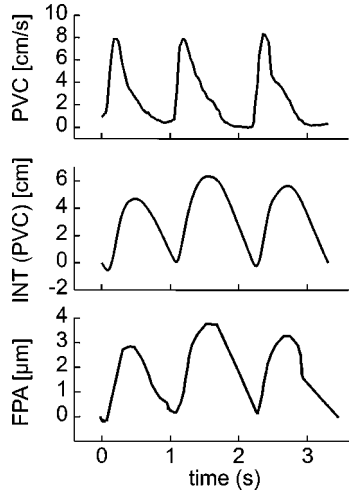

Fig. 1 A typical measurement as obtained in a healthy subject. The time course of pulsatile velocity component in the posterior ciliary arteries (PVC; upper panel), of the integrated pulsatile velocity component [INT (PVC); middle panel], and of fundus pulsation amplitude (FPA; lower panel) is shown.

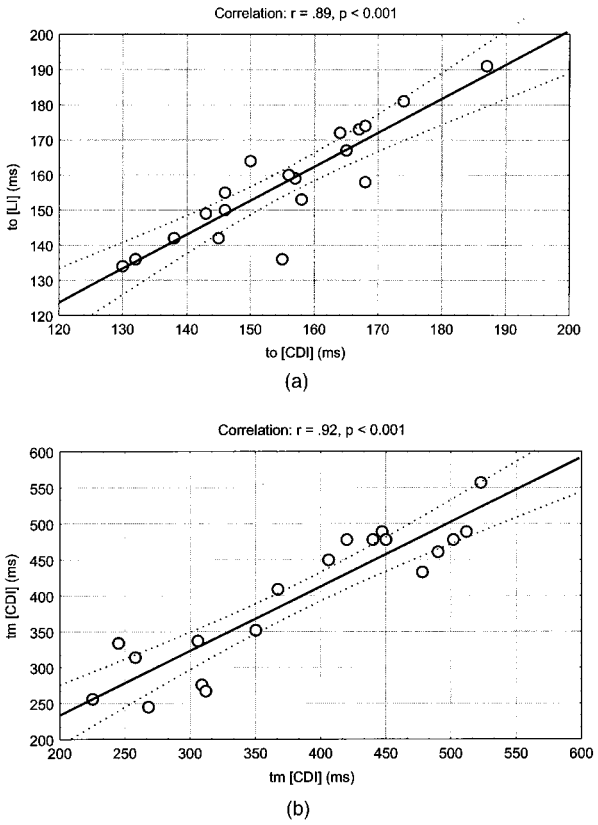

Fig. 2 Linear correlation ($n=19$) between the onset time (t_o = time from the r wave of the ECG to the onset of the pulse curve, upper panel) and the time of the maximum ocular volume (t_m = time from the r wave of the ECG to the maximum of the pulse curve, lower panel) as assessed from laser interferometry (LI) and the integrated velocity curve as measured with color Doppler imaging (CDI). The regression lines and the 95% confidence intervals are shown.

where BF_{vein} is the steady outflow of blood through the ocular veins. It is therefore possible to compare the onset time (t_o = time from the r wave of the electrocardiogram (ECG) to the onset of the pulse curve) and the time of the maximum ocular volume (t_m = time from the r wave of the ECG to the maximum of the pulse curve) from laser interferometric measurement of fundus pulsation and the integrated velocity curve as assessed with color Doppler imaging. A typical example for a velocity curve in the PCAs, the integrated velocity curve and the time course of ocular fundus pulsation is given in Figure 1.

These comparisons were done in 19 healthy young subjects with an ametropia of less than three diopters. Note t_o as assessed with laser interferometry was 158 ± 16 ms, whereas the same parameter as assessed from the integrated velocity curve was 155 ± 15 ms (not significantly different, paired t test). Note t_m was determined as 399 ± 97 ms by laser interferometry and as 385 ± 99 ms by color Doppler imaging (not significantly different, paired t test). Figure 2 shows that there is a high association between the parameters as assessed with these two methods, which argues that fundus pulsations are exclusively caused by the pulsatile inflow of blood through the PCAs.

4 APPLICATIONS

Laser interferometric measurement of FPA has so far been used to study the physiology, the pharmacology, and the pathophysiology of the choroidal circulation. As mentioned above we have shown that the choroidal circulation only slightly reacts to hyperoxia, whereas it is strongly dependent on arterial carbon dioxide tension.[51,52] Our results during isometric exercise, Valsalva maneuver, and increase in intraocular pressure indicate that the choroid has some autoregulatory capacity.[44,57] On the other hand even moderate physiologic stimuli may alter choroidal blood flow if they induce changes in breathing pattern and therefore changes in pCO_2.[44]

Intensive studies have been performed to elucidate the role of the *L*-arginine/nitric oxide (NO) system in the choroidal circulation. NO seems to be an important regulator of choroidal blood flow,[58,59] which is in keeping with animal experiments.[60,61] The ocular *L*-arginine/NO system is altered in patients with insulin-dependent diabetes mellitus,[59] which could contribute to the vascular complications associated with the disease. In addition, the choroidal vasodilator responses to insulin, histamine, and hypercapnia are dependent on NO production.[62–64] The endothelin system also plays an important role in the regulation of choroidal blood flow,[65,66] whereas the renin-angiotensin system seems to be less important.[67,68]

Further studies focused on the choroidal hemodynamic effect of therapeutic agents in ophthalmol-

ogy. Pentoxifylline increases choroidal blood flow after a single dose administration in healthy subjects[69] and after three months of therapy in patients with age related macular degeneration.[70] An extensive study has been performed on the effect of several antiglaucoma drugs on ocular hemodynamics in healthy subjects.[43] The results of this clinical trial indicate that topical administration of befunolol, metipranolol, timolol, clonidine, and dipivefrine slightly reduce choroidal blood flow. Ocular blood flow reduction was not observed with betaxolol, levobunolol, and pilocarpine.

Due to the high reproducibility and sensitivity of fundus pulsation measurement the technique is excellently suitable for pharmacodynamic studies, when only relative changes versus baseline are of interest. Whether FPA measurements can be used to compare different study groups is less clear. Nevertheless we have shown that FPA decreases with age[71] and that patients with proliferative diabetic retinopathy have reduced FPAs.[72] In addition, we have shown that age-related macular degeneration patients with classical neovascular membranes have locally reduced FPAs.[73]

5 CONCLUSIONS

Fundus pulsation amplitude can be taken as a relative measure of pulsatile choroidal blood flow in pharmacodynamic studies. In cases when a pharmacological or physiologic intervention induces significant changes in blood pressure or pulse rate the results have to be interpreted with caution.[55,67] Further studies are required to better define the exact relationship between changes in intraocular pressure, fundus pulsation amplitude, and pulsatile choroidal blood flow. In addition the ratio of nonpulsatile to pulsatile blood flow has not yet been determined.[74] Our recent results indicate that this problem might partially be overcome in pharmacodynamic studies, when changes in flow pulsatility are estimated from color Doppler imaging results.[67]

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