Pulse wave velocity in retinal arteries of healthy volunteers

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ABSTRACT

Background/aims Measurement of pulse wave velocity (PWV) in large vessels has been used extensively in clinical practice as an indirect measure of arterial stiffness and an indicator of cardiovascular risk factors. Arterial stiffness increases with age and in coronary artery disease. An in vivo clinical method to characterise arterial stiffness of the central microcirculation was developed.

Methods Time-dependent alterations of retinal vessel diameter were examined by the dynamic vessel analyzer in a randomly chosen eye of 10 young (26.0 (23.5, 27.0) years old (median (1st quartile, 3rd quartile)) and 10 old (67.0 (61.3, 69.5)) years old) healthy volunteers. Two segments of a retinal artery were measured simultaneously. The distance between the segments was measured using retinal photographs. The data were filtered and analysed using signal analysis methods in order to calculate PWV in the assessed retinal artery (rPWV).

Results rPWV differed significantly between young (21.5 (17.9, 4.6) mm/s) and old (243.8 (186.1, 347.7) mm/s) volunteers: (p=0.0001, Mann—Whitney test with Bonferroni correction).

Conclusions This study demonstrates a higher rPWV in elderly people than in young people. Therefore this new parameter resembles large artery PWV. This suggests that dynamic in vivo imaging of the central microcirculation enables the measurement of local microvascular stiffness with a commercially available medical device.

INTRODUCTION

The vascular system gradually stiffens because of the combined effects of ageing, high blood pressure and other vascular risk factors.[1] Large artery stiffness is non-invasively measured by pulse wave velocity (PWV).[2] The aortic PWV appears to be of high prognostic relevance for cardiovascular events.[3] Population- and patient-based cohorts demonstrated a strong association between increased aortic PWV and age,[4] coronary artery disease,[5] myocardial infarction,[6] heart failure,[7] stroke[8] and hypertension.[9]

By contrast, there are no microvascular measurements of stiffness. O'Rourke and Safar[10] hypothesised that cerebral microvascular disease results from the damaging forces of abnormal flow pulsations extending into small cerebral arteries as a consequence of arterial stiffening. The eye, with its unique property of direct non-invasive assessment of small retinal arteries, seems an ideal organ to gain insight in microvascular stiffness. Moreover, retinal arteries reflect the central microcirculation; they thus mirror the cerebral vasculature and could be associated with stroke. Finally, structural changes in the retinal vasculature are clinical predictors of systemic vascular disease.[11]

The present study was undertaken to assess the possibility of measuring PWV in retinal arteries (rPWV). Since age is an important factor influencing large artery stiffness, and hence PWV in large arteries increases with age,[1 2] we hypothesised in this study that rPWV increases with age similar to large artery PWV. We used a non-invasive approach of imaging a sequence of retinal vascular diameter changes with an ophthalmoscope. Data image and signal analyses were applied to determine rPWV.

MATERIALS AND METHODS

Subjects

Anamnestically healthy volunteers recruited at the Department of Ophthalmology at Munich University of Technology were enrolled in this study. One randomly chosen eye of each participant was measured. The volunteers were divided into two groups: 10 young subjects (six female, four male, age 26.0 (23.5, 27.0) years (median (1st quartile, 3rd quartile)) and 10 old subjects (seven female, three male, age 67.0 (61.3, 69.5) years). History of smoking, drug use and alcohol habits was recorded and an ophthalmic examination was performed in all subjects.

Inclusion criteria were: no history of systemic or ocular diseases; no systemic or topical medication, including contraceptive pills at the time of the examination, and no history of chronic medication; no history of smoking, drug or alcohol abuse; no cardiovascular events; visual acuity 0.8 or better; ammetropia with spherical equivalent within −3.5 and 3.5 D; astigmatism 1 D or less; intraocular pressure less than 20 mm Hg measured with Goldmann applanation tonometry; no pathological findings on slit lamp examination.

Measurements

Thirty minutes after pupil dilation with three consecutive drops of topical tropicamide (Mydriaticum Stulln; Pharma Stulln Ltd, Stulln, Germany) applied 10 min apart, continuous measurement of retinal arterial diameter was performed for 1 min using the retinal vessel analyzer (RVA; IMEDOS Systems, Jena, Germany). The fellow eye was covered during the examination in order to improve fixation of the volunteer. Blood pressure and pulse rate were recorded before and immediately after the measurement.

Properties of the RVA and its measurement principles have been described previously.[12] Briefly, the device allows non-invasive online assessment of

vessel diameter, depending on time and location along the vessel. For that purpose the RVA consists of a retinal camera (450 FF; Carl Zeiss, Jena, Germany), a CCD camera for electronic online imaging, and a personal computer for system control, analysis and recording of the data.

Off-line measurements

After the data acquisition, measurements of the arterial reactions at two distinct measuring vessel segments were taken off-line from videotape recordings with a novel version of the RVA called the dynamic vessel analyzer (DVA). In comparison to RVA, DVA has better measurement accuracy and allows simultaneous measurements of two vessel segments, which was crucial for the study. The peculiarities of DVA and its improvements compared with RVA have been described previously.[12 13]

For off-line measurement a template window reference box was positioned at a bifurcation or at a vessel intersection in the inferior or superior temporal part of the fundus one to three optic disc diameter away from the rim of the optic disc. Two temporal arterial segments—a proximal (1) and a distal one (2)—of approximately 300 relative units (RU) in length (corresponding to 300 μm in the Gullstrand's normal eye) were chosen within the template window for the simultaneous assessment (figure 1A).

Distance measurement

Standard fundus photographs showing the location of the measured vessel segments (figure 1A) were exported from the DVA program and then imported into VisualIS (IMEDOS Systems, Jena, Germany). The distance between the midpoints of the measured segments was assessed in pixels and recalculated in μm.

Data evaluation

A template with corresponding macros in a spreadsheet (MS Excel 2000) was created to filter, process and analyse the numerical data from the DVA and the VisualIS.

In order to filter and analyse the measured samples fast Fourier transform[14] (FFT) was used (an algorithm to compute the discrete Fourier transform and its inverse). The following common applications of FFT were used in the present study: frequency data filtering and cross-correlational analysis. A program based on FFT was created in MATLAB 7.0 (MathWorks Inc, Natick, Massachusetts, USA) for automated data processing.

From the whole temporal vessel diameter assessments lasting 1 min (figure 1B) data sequences were extracted for each subject for further analysis. Data sequences with no more than 0.5 s of discontinuity and less than 10% of whole data missing were analysed. Discontinuities of less than 0.5 s were filled by a macro program developed in MS Excel. Missed temporal points were linearly interpolated between the measured points.

Following preliminary tests on rPWV calculations from data sequences of different lengths extracted from 1 min assessments (128, 256, 512 and 1024 temporal points), a standard length of the analysed samples of 2^8=256 temporal points (≈10.2 s) was chosen for each subject, since FFT uses data samples of the length, which is a power of 2. This was a compromise between a higher precision of the evaluation, necessity of continuous analysed data sequences and reduction of the influence of heart rate variability

Both continuous analysed samples from original temporal vessel diameter assessments of a proximal (1) and a distal (2) arterial segment (figure 1) were filtered in two steps using band pass frequency filters. Both filtered samples assessed in (1) and (2) were slightly shifted to each other (figure 1C). Cross-correlation function of these filtered signals was calculated. Its absolute maximum showed the temporal phase shift between the assessed samples (figure 2).

The sampling rate of DVA assessment of 40 ms was insufficient for most measured temporal phase shifts in the present study. For more precise assessment of the time shift from the cross-correlation function, the latter was interpolated near the absolute maximum of the function with a polynomial[15] using MS Excel. The maximum of this fit was calculated and

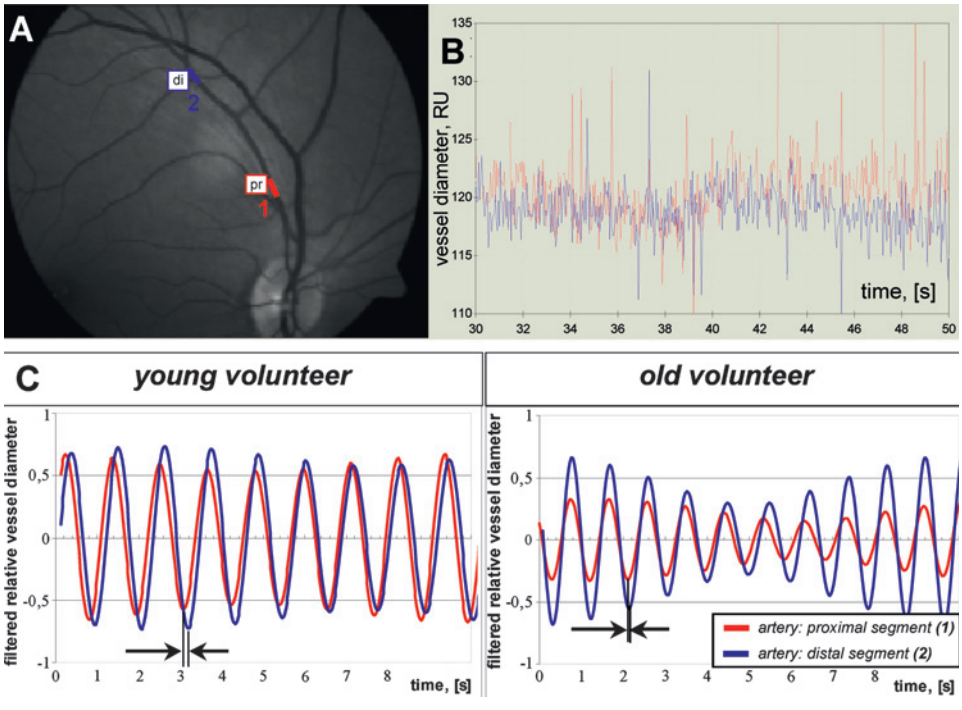

Figure 1 Continuous assessment and filtering of temporal vessel diameter changes in two retinal arterial segments: (1) proximal (red) and (2) distal (blue). (A) Location of measured segments in the fundus: the pulse wave propagates from 1 to 2. (B) Simultaneous dynamic vessel analyzer (DVA) assessment in segments 1 and 2: vessel diameters are measured in relative units (RU), which correspond to 1 μm in the Gullstrand's normal eye. Chaotic high frequency peaks of 5–20 RU in the time course due to eye blinks are eliminated using signal analysis. (C) Characteristic examples of filtered time courses as in (B). The red curve corresponds to the proximal arterial segment (1), and the blue curve pertains to the distal one (2). Since the pulse wave propagates from the proximal to the distal location, the blue curve is delayed compared with the red curve. This time delay is larger in a young volunteer than in a senior volunteer: a pulse wave needs more time for its propagation in an elastic retinal artery of a young volunteer than in a stiffer artery of an old volunteer. Thus the velocity of such propagation is lower in the young volunteer.

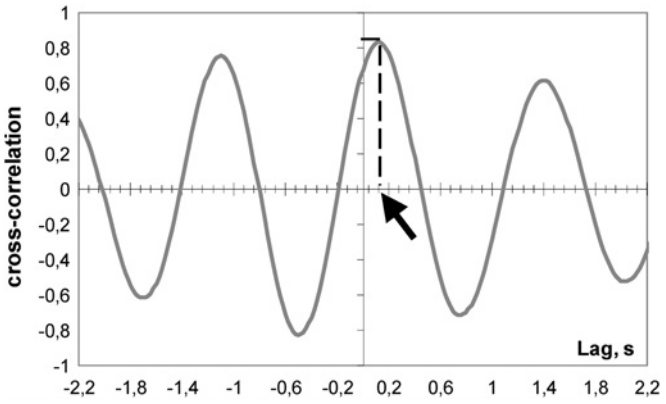

Figure 2 Cross-correlation function of the filtered time courses shown in fig 1C, left panel. A lag in the maximal point of the cross-correlation function (black arrow) equals 0.12 s and corresponds to the phase shift between both filtered time courses. The lag is positive, since the first signal from the proximally located arterial segment has been assessed before the second one from the distally located segment.

considered as the absolute maximum of the cross-correlation function, with the corresponding temporal lag being the desired phase shift between vessel diameter assessments in segments 1 and 2 (figure 1).

Calculation of rPWV

Retinal PWV was calculated in mm/s for each individual case using the conventional formula:

$$\text{rPWV} = \text{distance between analysed segments} / \text{phase shift between vessel diameter assessments.}$$

Since the value of rPWV depends on absolute vessel diameter, we also calculated diameter-independent normalised rPWV (rPWV_n) values in 1/s as:

$$\text{rPWV}_n = 2 \times \text{rPWV} / [\text{vessel diameter(place1)} + \text{vessel diameter(place2)}].$$

Statistical analysis

Because it was impossible to prove normal distribution of measurement data, non-parametric statistics were applied for the evaluation. Values of measured and calculated parameters are represented in the following form: median (1st quartile, 3rd quartile). Both groups were compared by Mann–Whitney test. Parameters within the groups were compared by Wilcoxon test. Due to the small number of subjects, non-parametric tests were applied on the level of significance of p=0.05 for each evaluated parameter. Non-parametric statistics were calculated by MS Excel, SPSS and by Primer of Biostatistics, v. 4.03 by Glantz.[16]

In order to avoid errors in multiple testing, Bonferroni correction[16] was used to report significant differences between the groups.

RESULTS

Principal results of the study are shown in the table 1. p Values in the table 1 are represented as local values as a result of group comparison for each parameter.

Circulation parameters

The mean systemic blood pressure and the pulse differed significantly between groups at the beginning of the examination (p=0.00007; table 1; p=0.0009 after Bonferroni correction). These circulation parameters did not change significantly within any group during the examination (p=0.45).

Pulse and cardiac period values calculated during mathematical analysis of DVA assessments did not differ significantly between the groups (p=0.105; table 1). However, the young volunteers showed higher variability of pulse and period within the group than the elderly.

Table 1 Principal results of the study

	Median (1st quartile, 3rd quartile)		
	Young volunteers (n=10)	Elderly volunteers (n=10)	Significance (local p Value)
Age (years)	26.0 (23.5, 27.0)	67.0 (61.3, 69.5)	0.00001
Mean systemic blood pressure (mm Hg)	79.7 (74.1, 82.9)	100.3 (93.4, 112.7)	0.00007
Systemic pulse rate (1/min)	59.0 (55.0, 60.0)	68.5 (61.0, 72.8)	0.105
Arterial diameter, proximal segment (μm)	105.5 (98.3, 115.7)	109.8 (105.4, 124.4)	0.290
Arterial diameter, distal segment (μm)	104.8 (98.6, 116.5)	112.2 (100.9, 126.5)	0.545
Pulse rate (retinal arteries) (Hz)	0.98 (0.90, 1.12)	1.07 (1.07, 1.15)	0.096
Period (retinal arteries) (s)	1.02 (0.90, 1.11)	0.93 (0.87, 0.93)	0.096
Segmental distance (μm)	6475 (5537, 6919)	6671 (6141, 7139)	0.496
Segmental phase shift (s)	0.30 (0.18, 0.37)	0.03 (0.02, 0.04)	0.00002
Segmental phase shift (°)	134 (48, 144)	11 (7, 14)	0.00002
rPWV (mm/s)	21.5 (17.9, 34.6)	243.8 (186.1, 347.7)	0.00001
rPWV_n (1/s)	424 (312, 709)	4380 (3431, 5345)	0.00001

rPWV, retinal pulse wave velocity; rPWV_n, normalised retinal pulse wave velocity.

Assessment of retinal arterial parameters and rPWV

The average diameters of both measured arterial segments differed neither between the proximal and distal location in both groups (p=0.350; p=0.450) nor between the groups (p=0.290; p=0.545; table 1). This fact substantiated the comparison rPWV values between the groups.

Young healthy volunteers showed a significantly larger phase shift between filtered oscillations of retinal arterial diameter than old volunteers (table 1 and figure 1C; p=0.0003 after Bonferroni correction). Thus, in the retinal arteries of young volunteers the pulse wave needs more time to propagate between the measured segments than in arteries of old volunteers. Locations of assessed vessel segments and distances between them were determined only by the structure of the vessel tree and did therefore not differ between the groups (p=0.496; table 1).

rPWV values calculated from the phase shift and the distance between measured vessel segments differed tenfold between the groups (p=0.0001 after Bonferroni correction) and amounted to 21.5 (17.9, 34.6) mm/s in young volunteers and to 243.8 (186.1, 347.7) mm/s in old volunteers (table 1 figure 3, left panel). rPWV_n amounted to 423 (312, 709) 1/s in young and to 4380 (3431, 5345) 1/s in old volunteers (p=0.0001 after Bonferroni correction).

DISCUSSION

This is the first report of quantitative values of rPWV using a non-invasive, commercially available device. We demonstrated that rPWV is low in young and high in old volunteers, potentially reflecting age-related vascular stiffness as previously described for large arteries.[2]

Historically the assessment of vascular pulsations in the eye has been limited to the choroidal circulation.[17 18] Assessment of

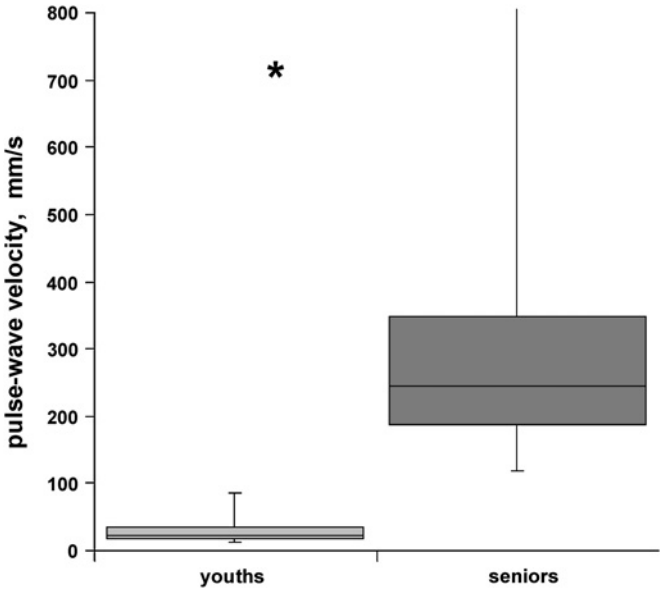

Figure 3 The value of the calculated parameter retinal pulse wave velocity (rPWV) differs significantly between both groups: p=0.0001, Mann–Whitney test with Bonferroni correction. These results show increased retinal arterial stiffness in old healthy volunteers compared with young volunteers.

pulsations of the blood flow in a single location, for example in the optic nerve head, retinal and choroidal circulation, is possible using the method described by Petrig *et al*.[19] Pulse wave propagation from the heart to the ophthalmic artery and choroidal circulation has been estimated at 4.08 m/s in a study of healthy subjects by Michelson *et al*.[20] Indirect measurements of retinal vessel pulse are complicated by the fact that the choroidal circulation constitutes most of the ocular blood flow[21] and determines most of the pulse amplitude.[18]

PWV in the microvessels was observed to be two orders of magnitude slower than in the aorta.[22] These experimental findings were shown to be consonant with linear pulse wave transmission theory in a branching system of vessels.[23] Thus the rPWV values reported in the present study seem to be consistent with contemporary vascular physiology.

In 2000 Otto reported quantitative data on PWV in retinal arteries by analysis of motion-compensated angiography image sequences.[15] Otto reported rPWV values of 24–26 mm/s in retinal arteries of a healthy 30-year-old volunteer, 240 mm/s in a healthy 63-year-old volunteer and >300 mm/s in a 45-year-old patient with arterial hypertension.[15] These estimated rPWV values are in accordance with our measurements.

The main limitations of the method of Otto are its invasiveness and the need for correction of the sampling rate of the laser scanning system. By contrast, our model is non-invasive and reproducible based on a commercially available DVA system.[12]

Using a DVA system Gugleta *et al* recently analysed phase delay between retinal arterioles and venules and used it to calculate choroid-to-retina pulse delay.[13] In their study healthy young vasospastic subjects demonstrated an altered phase delay between the arterioles and venules, presumably indicating faster pulse wave propagation in the retinal arteriolar tree. However, rPWV was not calculated.

Considerations of the technical features and limitations of our method must be elucidated to enable better interpretation of our reported results.

Limitations in the image resolution of DVA and a corresponding sampling rate of 25 measurements/s restrict rPWV assessment to a limited range. Assuming the distances between two measured arterial segments in the range of 4.5–9.5 mm, as in the present study, one can derive the maximal measurable rPWV value as being in the range of 112.5–237.5 mm/s. In addition, inaccuracy of rPWV measurements from raw DVA data increases with the measured value. Calculating with a standard formula for indirect measurements for the distance of 5 mm between the measured arterial segments, the inaccuracy of rPWV values would amount to: ±0.8 mm/s for rPWV=10 mm/s (ie, ±8%), ±20 mm/s for rPWV=50 mm/s (±40%) and ±80 mm/s for rPWV=100 mm/s (±80%). In the present study we used mathematical signal processing of raw DVA data. This allows measurement of rPWV values up to 5000 mm/s and attainment of higher measurement accuracy, which amounts to about ±4.0% for rPWV=20 mm/s, ±4.5% for rPWV=100 mm/s and ±10.8% for rPWV=500 mm/s.

One potential limitation is that one randomly chosen retinal artery was chosen to assess rPWV. Since the whole retinal vasculature undergoes age-related changes in vessel structure and biomechanics, we assumed that rPWV values in all large retinal vessels of a healthy subject are of the same order and depend on the vessel diameter and other factors. We tested this assumption in some eyes (see an example in figure 4) and concluded that it is reasonable. However, the reliability of the assumption needs to be elucidated in future studies.

As shown in figure 4, some retinal artery segments are almost straight, whereas others are not. Arterial tortuosity may influence retinal PWV and needs to be elucidated in future studies, particularly as vascular disease is often associated with arterial tortuosity.

Aortic PWV usually increases by 30–50% from 20 to 70 years of age.[2 24] In the present study an almost tenfold increase in retinal PWV for the similar age range was found. There is no direct information in the literature that can confirm or reject this measured tenfold age-related increase of rPWV. Since small arteries are shown to be much more elastic than large arteries,[23]

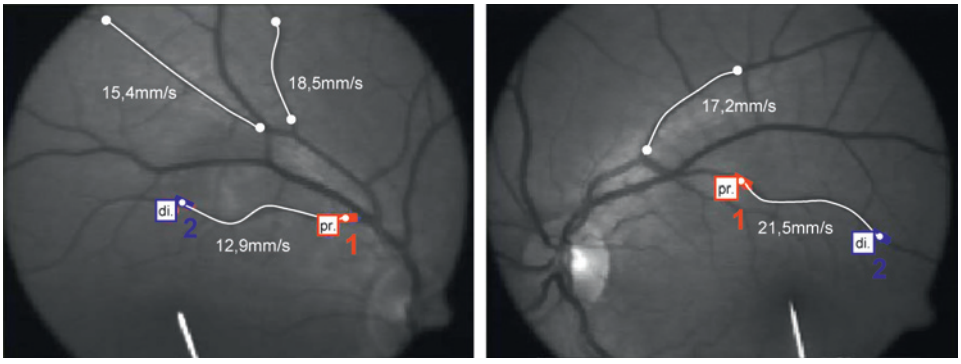

Figure 4 Pulse wave velocity (PWV) values in different arterial segments of both eyes of a young healthy volunteer are of the same magnitude. Since the retinal PWV values are dependent on the absolute vessel diameter, the corresponding normalised retinal PWV ($rPWV_n$) values are even more stable. $rPWV_n$ in this example are 308, 293, 286, 330 and 312 1/s (counter clockwise, starting from the left upper corner correspondingly) with a coefficient of variation of 5.6%.

which predict corresponding rPWVs being in the range of cm/s, it cannot be excluded that the ageing process in small arteries differs from this process in large arteries.

In this first report on rPWV assessment using RVA we tested the method in a small cohort of anamnestically healthy volunteers in order to reveal a possible ageing effect on the retinal PWV values and to estimate their normal range. The broad range of retinal PWV in the elderly subjects may reflect a broad band of cardiovascular risk factors in this group compared with the examined young group, rather than the decrease of measurement accuracy of the method at high rPWV values. However, in this pilot study neither intersession nor interobserver variabilities were measured. Thus, the broad range of rPWV values in elderly subjects may reflect the case of measurements variability.

Further studies with a more detailed description of the cardiovascular risk factors of the examined participants need to be performed in order to give a perspective to the clinical usage of the method and to reveal its potential value for differentiating healthy from pathological values of retinal PWV.

In summary, we propose a method to investigate rPWV. Our method is based on the phase delay between retinal vessel pulsations at different places along a measured vessel, which is similar to common approaches for PWV measurements in large vessels.[24] Our results are in accordance to theoretical considerations of PWV in microvessels and in accordance with invasive measures of retinal PWV. Finally, we show an age-related increase in retinal PWV, suggesting that ageing of retinal arteries is associated with vascular stiffness similar to large arteries. Whether retinal PWV mirrors the cerebral vasculature and is associated with cerebrovascular disease needs to be examined in further studies.

Competing interests None.

Patient consent Obtained.

Ethics approval This study was conducted with the approval of the Ethics Committee of the Klinikum rechts der Isar (Munich University of Technology). All procedures adhered to the tenets of the Declaration of Helsinki.

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