Title: Structural characteristics of the optic nerve head influencing human retinal venous pulsations

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Abstract

The relationship between structural characteristics of the optic nerve head and venous pulsations in the human eye remain unknown. Using photoplethysmographic techniques we investigated whether properties of the human retinal veins and their surrounding structures influence venous pulsation. 448 locations of venous pulsation were analysed from 26 normal human eyes. Green channel densitometry derived from video recordings of venous pulsations were used to generate a map of venous pulsation amplitudes along retinal veins. Optical coherence tomography was used to perform quantitative measurements of tissue characteristics at sites of high and low amplitude points as well as in a second analysis, at maximal amplitude pulsation sites from superior and inferior halves of the eyes. Structural characteristics measured included venous diameter, distance from pulsation point to cup margin, vessel length from pulsation point to vein exit, tissue thickness overlying vein, optic disc diameter and presence of a proximal arteriovenous crossing. Increasing venous pulsation amplitudes were associated with larger applied ophthalmodynamometry force, increasing venous diameter, and decreasing absolute cup margin distance (all $p < 0.001$). Increasing distance of maximal amplitude pulsation point to cup margin was associated with the presence of a proximal arteriovenous crossing, increasing venous diameter, and decreasing tissue depth (*all* $p \leq 0.001$). Venous diameter and tissue depth alter venous compliance, which is likely to be a major factor determining sites of venous pulsation.

Key words: Retina; OCT; glaucoma; venous pulsations; optic disc; photoplethysmography; optical coherence tomography; optic nerve head

1. Introduction

Retinal venous pulsations occur spontaneously in up to 95% of normal human eyes.[1] They are visualized as an oscillation of the retinal vein wall and show a variation in location, generally occurring around the optic disc surface close to the venous exit through the lamina cribrosa.[2] The clinical importance of this feature is its ability to indicate the health of the retinal circulatory system and the absence of vein pulsation is linked to numerous disease states such as glaucoma and retinal vein occlusion.[1, 3] Furthermore, absence of venous pulsations may be useful in the diagnosis of raised intracranial pressure.[2] When absent, venous pulsations may be induced by increasing intraocular pressure (IOP) through application of ophthalmodynamometric force (ODF) to the globe which may be recorded as a venous pulsation pressure.[4] Though there appears to be a strong relationship between venous pulsation pressure and IOP, this correlation is not well defined. Our previous studies demonstrated the relationship of retinal vein pulsation timing to phases of intracranial pressure, implicating cerebrospinal fluid pressure (CSFp) pulsation as the prime generator with the pulse wave moving along the vein in a retrograde manner.[5, 6]

Though retinal vein pulsation is often limited to a small segment of the vein, there is vast inter-individual variation in the location of this pulsation.[2] Determinants of such variation in not only pulsation location but also occurrence have not yet been identified. It is postulated that structural factors in the optic nerve head (ONH) inherent to the retinal veins may influence venous pulsation. Empirical and modelling work identifies elevated vessel wall compliance as a factor reducing pulse wave velocity and distal amplitude, which occurs when veins are larger and have less rigidity.[6, 7]

The structure of the optic disc and disc-vessel configuration may also be significant to the distribution of intravascular pressure gradient along the retinal vein.[2] The ONH consists of densely packed tissue with complex cellular relationships consequent to the intricate embryological processes involved in its development.[8, 9] Investigating relationships between these tissues may help determine possible influences to retinal venous pulsation. Additionally, analysis of pulse waves along vessels may provide information regarding a

possible pulse-generating site, such as the right atrium of the heart producing a jugular venous pulse.[6, 10]

Quantitative measurements of ONH characteristics have been reliably measured in human eyes by spectral domain optical coherence tomography (SD-OCT).[11] Our recently published photoplethysmographic technique allows us to accurately determine the location and amplitude of venous pulsation at varying locations surrounding the human optic nerve.[12, 13] In this study, we correlate venous pulsation with SD-OCT anatomical parameters to quantitatively and qualitatively study structural characteristics surrounding sites of venous pulsation.[14]

2. Materials and Methods

2.1 Ethics

This study was approved by the Human Research Ethics Committee of The University of Western Australia. All patient imaging was performed at the Lions Eye Institute in Perth. The study was conducted in accordance with the tenets of the Declaration of Helsinki and informed consent was obtained from subjects with explanation of the nature and possible consequences of the study.

2.2 Volunteers

Images were acquired from both eyes of 16 human volunteers, aged between 22 and 69 for this study. Human volunteers had no documented history of eye disease. Intraocular pressure measurements were performed along with baseline fundus photo and 24-2 Humphrey visual fields with SITA standard to exclude the presence of ocular conditions. One subject had systemic hypertension treated with a single antihypertensive agent (Ramipril). All other subjects had no past medical history or medications. A mydriatic agent was used prior to imaging. The demographic data of each subject are presented in Table 1.

2.3 Spectral Domain OCT Imaging

Spectral domain OCT (Spectralis SD-OCT, Heidelberg Engineering, Inc., Heidelberg, Germany) was performed on both eyes of volunteers. Details of the principle of SD-OCT have been previously described.[15] Images encapsulated a region 15 x 10 degrees centred on the optic disc with 49 sections acquired in both the horizontal and vertical planes. 30 images were averaged per plane and Enhanced Depth Imaging was utilized to maximize image quality.

2.4 Venous Pulsation Recordings

Ophthalmologists (W.M. and A.R.) performed video recordings of venous pulsations as described by Morgan et al.[16] A slit lamp camera recorded video and sound signals from a pulse oximeter placed over the subject's right index finger. Video resolution was 1920 × 1080 pixels at 25 frames per second. All timing of the signals was made in relation to the frame count and so a precision of 0.04 seconds was possible.

Retinal vein pulsation was recorded with a 60-diopter non-contact lens. Following this, an ophthalmodynamometer (Meditron, Voeklingen, Germany) using a contact lens surrounded by a ring force transducer was applied to the eye using contact gel after local anaesthetic application. Graded forces of varying ODF were applied to aim for target pressure ranges of 0 (i.e. at spontaneous venous pulsation) and between forces of 1-15, 15-30, 30-45 for each eye. Video recordings were taken of venous pulsations in the hemiveins and central retinal vein.

2.5 Image Preparation

Our previous technique of creating densitometry maps was utilized.[13] In short, video recordings of venous pulsation over three cardiac cycles were imported into Adobe Photoshop CS5.1 as individual frames. A calibrated algorithm utilizing green channel densitometry measurements that reflect haemoglobin absorption timed to cardiac cycle was used to calculate a densitometry map reflecting the amplitude of pulsation at individual locations.

2.6 Quantification of Tissue Parameters

Quantitative data from corresponding SD-OCT images were attained following identification of venous pulsation recordings with our photoplethysmographic technique. Specifically, venous pulsation points were carefully co-localized between densitometry maps, baseline fundus photos and simultaneous confocal scanning laser ophthalmoscopy fundus imaging on the Spectralis imaging platform. This allowed interpolation of topographic and tomographic images at selected points of interest.[17] Characteristics of veins at high and low amplitude pulsation points, and surrounding tissue characteristics were recorded using defined histologic parameters.[18,19] Measurements of these characteristics were made for noncontact recordings (i.e. no ODF applied) and then at three graded levels of applied ODF. Measurements were performed using commercial software (Universal Ruler 3.6) calibrated with the Spectralis SD-OCT generated scale bars to allow manual quantification of tissue characteristics for each eye (Figure 1):

- Venous diameter: The maximum chord axis between the inner vein walls using the interface of blood/lumen and vessel wall on cross sectional imaging.[18]

- Tissue depth: The minimum perpendicular distance from the outer edge of the vein wall to the inner limiting membrane on cross sectional imaging.[18]

- Optic disc diameter: The maximum distance between Bruch membrane opening on OCT horizontal cross sectional scan series.[19]

- Arteriovenous (AV) crossing: The presence of an artery overlying a vein proximal to the site of venous pulsation.

- Central relationship: Whether the point of venous pulsation was superior or inferior in relation to the horizontal equator of the cup.

- Cup margin distance: The shortest direct distance from pulsation point to the cup edge on the optic nerve head surface on fundus imaging. A positive numeric measurement was assigned to pulsation points peripheral to the cup edge. A negative numeric measurement was assigned to points located central to the cup edge.

- Absolute cup margin distance: The absolute numerical value denoting the shortest direct distance from pulsation point to the cup edge on the optic nerve head surface. This was irrespective of proximal or distal relations of the pulsation point to the cup edge on fundus imaging.

- Maximal pulsation distance: The distance from cup margin to point of maximal venous pulsation measured on photoplethysmographic densitometry. A maximal pulsation point was identified from both the superior and inferior half of each densitometry map.

- Venous segment length. The distance following the entire trajectory of the vein, from location of venous pulsation to its exit at the optic nerve head on fundus imaging.

2.8 Statistical Analysis

All data are expressed in terms of mean and standard error calculated using Sigmastat (Sigmastat, ver. 3.1; SPSS, Chicago, IL). Multiple measurements from right and left eyes of the same individual were analyzed using R (R Foundation for Statistical Computing, Vienna, Austria). First, linear mixed modeling was performed to test associations between response variable: pulsation amplitude, and explanatory variables: absolute cup margin distance, venous segment length, venous diameter, tissue depth, optic disc diameter, AV crossing, central relationship, age, and sex. A second linear mixed model used distance to points of maximal pulsation amplitude in both upper and lower hemiveins as a response variable (cup margin distance or venous segment length), with

variable ODF and other tissue factors as explanatory variables. Sequential elimination of non-significant variables was utilized. Akaike Information Criterion (AIC) values were calculated to determine the preferred response variable (cup margin distance or venous segment length) for the second linear mixed model. The models used, included random effects of "Right" or "Left" nested within "eye donor" to account for correlation between multiple measures from each eye and between right and left eyes.[20] To comment on the intra-individual differences of the determinants of retinal venous pulsations, we calculated the intraclass correlation coefficients (ICC) for the 2 linear mixed models to estimate the ratio of variance found in the data clusters from individual patients to the overall variance.

2.9 Measurement Reproducibility

To assess the inter-observer variation in SD-OCT measurements, a reproducibility study was performed on six eyes of six different subjects. This was quantified on a separate occasion by a second observer. Repeated measurements were performed on venous diameter, tissue depth, optic disc diameter and cup margin distance at areas of high amplitude venous pulsation for varying levels of applied ODF. For reproducibility measurements, the coefficient of variation was calculated for each parameter. It was calculated by expressing the standard deviation divided by median multiplied by 100, as a percent.

3. Results

3.1 Human Volunteers

The mean age of volunteers was 36.06 ± 3.88 years. We examined 13 right and 13 left eyes from a total of 16 volunteers (11 males and 5 females). Six eyes of six volunteers were excluded due to motion artefact and limited tolerability to ophthalmodynanometry applanation. All subjects had intraocular pressure measurements less than 20 mmHg bilaterally and full visual fields with reliable indices. A total of 64 video clips were analysed.

3.2 Morphometric tissue characteristics predicting amplitude of venous pulsations

A total of 448 venous pulsation locations were analysed. The amplitude data was normally distributed when checked with quantile-quantile plots. Increased venous pulsation amplitudes were associated with larger ODF (slope = 0.105 units per 1g ODF; 95% Confidence Interval [CI], 0.052 to 0.163, $p < 0.001$) and larger venous diameter (slope = 0.035 units per µm; 95% CI, 0.019 to 0.056, $p < 0.001$, Figure 2). There was no significant correlation of venous pulsation amplitude to age, tissue thickness, optic disc diameter, AV crossing or relationship to the centre of the disc (all $p > 0.134$).

Absolute cup margin distance was also associated with increased venous pulsation amplitudes (slope = -0.009 units per µm; 95% CI, -0.012 to -0.008, $p < 0.001$), as was venous segment length (slope = -0.008 units per µm; 95% CI, -0.009 to -0.006, $p < 0.001$). Given the high correlation between these tissue parameters (correlation coefficient of 0.834), the preferred variable was determined by AIC values. Absolute cup margin was chosen over venous segment length (*AIC* = 3142.397 compared to *AIC* = 3167.706 respectively

3.3 Morphometric tissue characteristics associated with point of maximal venous pulsations

Two points of maximal pulsation were included for analysis on each densitometry map - one located superior and one inferior to the centre of the optic disc. A total of 196 maximal amplitude pulsation points were identified. Maximal pulsations were located 166.445 µm more distally from the optic cup margin (95% CI, 82.665 to 284.475, $p = 0.001$) when associated with a proximal AV crossing (Figure 3). At points of maximal venous pulsation, a larger venous diameter (slope = 2.322 µm per 1 µm diameter; 95% CI, 1.104 to 3.528, $p < 0.001$) and decreased tissue depth (slope = -2.487 µm per 1 µm thickness; 95% CI, -3.287 to -1.461, $p < 0.001$) were correlated with increased distance (Figure 4). There was no significant correlation of maximal pulsation point cup margin distance with age ($p = 0.754$) or optic disc diameter ($p = 0.863$) or whether pulsation was in the superior or inferior optic disc ($p = 0.279$).

3.4 Measurement Reproducibility

220 measurements were performed on 55 venous pulsation points from six eyes of six different subjects. These were quantified on a separate occasion by a different observer. The reproducibility measurements with this technique demonstrate a median coefficient of variation of 20.028% for measuring cup margin distance, 12.389% for venous segment length, 24.513% for tissue depth, and 9.959% for venous diameter.

3.5 Intraclass Correlation Coefficients

The first linear mixed model assessing effects upon pulsation amplitude (finding association with ODF, cup margin distance and vein diameter) found an intraclass correlation coefficient (ICC) within patients of 0.21, and the similar model assessing effect of vein segment length instead of cup margin distance found an ICC of 0.23. The second linear mixed model assessing effects upon cup margin distance to the point of maximum amplitude (finding association with AV crossing, vein diameter and tissue depth) found an ICC within patients of 0.55.

4. Discussion

We observed a strong relationship between venous pulsation amplitude with absolute cup margin distance and venous segment length. The optic cup margin as a peak location of venous pulsation amplitude may suggest an effect of wave reflectance in this area. A vein crossing the cup margin undergoes an upwards-sloping venous trajectory. A change in trajectory may cause the pulse wave generated retrogradely from the retrolaminar portion of the vein under the influence of cerebrospinal fluid pressure to reflect off the vein wall.[21,22] This reflection can add to the incoming wave increasing pulse amplitude at or proximal to the cup margin and may explain the occurrence of maximal pulsation at this location. It has been observed amongst glaucomatous eyes, where the optic cup is deeper

and the vein changes direction at an even more pronounced angle, that venous pulsation is typically seen at the edge of the rim or just inside the optic cup.[23]

Theoretically, venous pulsation amplitude is highest at sites of high venous compliance which attenuates pulse waves distally along a vessel.[24] This is consistent with our observations that larger venous diameters are associated with higher venous pulsation amplitude. Similar results have been observed in studies of the rat eye retinal veins, where in vivo high speed imaging systems identified veins of larger diameter being associated with larger pulsation amplitudes.[25]

It is of interest that points of maximal pulsation were associated with proximal AV crossings. An AV crossing compresses the vein and reduces venous wall mobility and compliance. Low compliance, more rigid vessels transmit pulse waves with less attenuation to regions where larger pulsation is seen.[24] The association of decreased tissue thickness at the location of maximal venous pulsations may also be explained by high compliance, with thinner overlying tissue allowing greater compliance and allowing greater venous pulsation. We chose to measure minimum perpendicular distance from the outer edge of the vein wall to the inner limiting membrane of the retina as it may better reflect any tissue compression effect upon the vessel. There is likely to be an interaction between factors that alter venous compliance and the location of maximal venous pulsation.

We acknowledge several limitations of our study. Our photoplethysmographic technique has inherent imprecision with a coefficient of variation of 13% for vessel pulsation amplitude and 4% for pulsation timing.[16] It is also possible that venous pulsation amplitudes may be partially enhanced by artefact at the cup margin. Our technique video records the green-channel light absorption of haemoglobin to calculate pulsation amplitude, and so an upwards sloping vein at the cup margin has a broader blood column facing the camera, which may appear as increased light absorption and higher amplitude pulsation on subsequent analysis.[12] Consideration should be given to the degree of precision achievable on SD-OCT which allows an optical resolution of approximately 7μm in depth (axial resolution) and 14 μm transversally (lateral optical resolution).[26]

These measurements may also be influenced by eye length and refractive errors. In particular, measurement of tissue depth and other axial measurements may be under-represented as axial length increases.[27] In addition, when measuring vessel diameters from OCT scans the posterior vessel wall is often not easily visible. This study used readily available anatomical measures related to retinal veins on the optic disc. We did not have access to, and did not perform, complex analysis of vessel geometry. Geometric analysis may allow better modelling of wave reflection and local vessel compliance variation.

5. Conclusions

We have identified that some major factors inherent to retinal veins, as well as their relationship to surrounding optic disc structures, may influence venous pulsation characteristics. Factors that alter venous compliance, such as venous diameter, presence of AV crossings, and tissue depth, affect sites of venous pulsation. Our observations support the hypothesis that at areas of high compliance, pulse wave amplitude tends to be maximised, and that there is attenuation of pulse waves further along a more compliant vessel. It may be useful to investigate how changes in venous compliance may affect venous pulsations in various retinal vascular diseases like diabetic retinopathy and venous occlusive disease, as well as glaucoma.

TABLE 1. Patient Demographic Details

Patient	Age (y)	Sex	Eyes Analysed	Past Ophthalmic History
A	50	F	R + L	-
B	29	M	R + L	-
C	27	M	R + L	-
D	53	M	R + L	-
E	30	M	R + L	-
F	22	M	R + L	-
G	29	F	L	-
H	38	M	L	-
I	48	F	R	-
J	22	M	R	-
K	63	M	L	-
L	25	M	R	-
M	69	M	R + L	-
N	23	F	R + L	-
O	23	M	R + L	-
P	26	F	R + L	-

Legends

Figure 1 – Methodology for quantification of anatomic measurements. Schematic diagram of an optic nerve head (A) and corresponding optical coherence tomography cross section at site of high amplitude venous pulsation (B). Retinal veins (blue) and arteries (red) were identified. Cup margin distance (dashed green line), venous segment length (dashed red line) and presence of arteriovenous crossing proximal to site of venous pulsation were noted (black arrow). Tissue depth (solid green line) and venous diameter (dashed yellow line) were measured. Scale bar = 200 µm.

Figure 2 – Comparison of venous diameter measurements. Representative optical coherence tomography en face view of optic nerve head (A) and corresponding cross sectional imaging (B) for three locations. Larger venous diameter was associated with larger amplitude pulsation. Location (1) shows pulsation amplitude of 2.1 units with venous diameter of 86 µm, 17.6 units with 156 µm (2) and 12.7 units with 133 µm (3). Scale bar = 200 µm.

Figure 3 – Effect of arteriovenous (AV) crossing on venous pulsation location. Optic nerve head photo (A), video frame (B) and corresponding pulse amplitude map (C). Pulsation point (1) shows an increased cup margin distance associated with the presence of a proximal AV crossing (black arrow) compared to a pulsation point without a proximal AV crossing (2).

Figure 4 – Effect of venous diameter and tissue depth on venous pulsation location. A pulse amplitude map of high amplitude venous pulsation points (A), corresponding en face optical coherence tomography (OCT) image (B) and OCT cross section (C). Increased cup margin distances are associated with increasing venous diameter (yellow line) and decreasing tissue depth (green line). Point (1) with a small cup margin distance has a venous diameter of 123 µm and tissue depth of 148 µm compared to point (2) which has a venous diameter of 172 µm and tissue depth of 25 µm. Scale bar = 200 µm.

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