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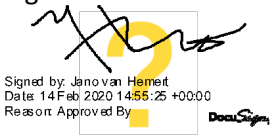
Dr Jano van Hemert Optos plc Queensferry House Carnegie Campus Enterprise Way, Dunfermline, Scotland, UK KY11 8GR	Signature and date 

Primary Objective

Arterial to venous ratio (AVR) measurements from the left eye (semi-automated and automated) will be investigated against clinic blood pressure (average of 4 measurements) for performance of classification of hypertensive status (based on gold standard; daytime mean ambulatory systolic blood pressure).

Dependent variable

Hypertensive status. Hypertension defined as daytime average ambulatory systolic and/or diastolic blood pressure (ABPM) measurement greater than or equal to 135/85 mmHg, respectively.

Independent variables

AVR; semi-automated and automated measurements in both right and left eyes. These measurements are obtained using computer code, please see appendix for details of the method. Also in the appendix are the secure hash algorithm (SHA) checksums of the code and results, which enables anyone to verify the code that is used is exactly the same as when the checksum was created.

Clinic blood pressure (CBP); average of four readings, two per arm.

Primary analysis

Demographics of the sample will be described. Descriptive statistics for quantitative parameters will be presented as mean, standard deviation (SD); or median and inter-quartile range (IQR) for skewed data.

Simple comparison analyses will be performed between those with and without hypertension, and those who are under treatment for hypertension vs those who are not, for each of the included variables. Univariate analyses will be completed using either a Pearson chi-square (for categorical variables) or independent samples t-test (for continuous variables). A p value of < 0.05 will be used to demonstrate a statistically significant difference.

Receiver operating characteristic (ROC) curves and area under the curve (AUC) estimates will be estimated for the prediction of hypertensive status.

In the primary analysis AVR measurements from the left eye (semi-automated and automated) will be investigated against clinic BP (average of 4 measurements) for performance of classification of hypertensive status (based on gold standard; daytime mean ABPM) measured by AUC. We have pre-specified hierarchical primary endpoints in the table below;

Exclusion criteria

The following data will be excluded from the analysis:

- Insufficient ABPM measurements; less than 70% successful daytime readings.
- Retinal image of inadequate quality for determination of AVR as defined in the appendix.

Step 1	Non-inferiority of semi-automated AVR measurements from the left eye (where unavailable, measurements from the right eye will be used) against CBP for predicting hypertensive status in the full sample where semi-automated AVR measurement is defined. If significant at $p < 0.05$ move on to Step 2
Step 2	Non-inferiority of automated AVR measurements from the left eye (where unavailable, measurements from the right eye will be used) against CBP for predicting hypertensive status in the full sample where automated AVR measurement is defined. If significant at $p < 0.05$ move on to Step 3
Step 3	Non-inferiority of semi-automated AVR measurements from the left eye (where unavailable, measurements from the right eye will be used) against CBP for predicting

	hypertensive status in the untreated sample where semi-automated AVR measurement is defined. If significant at p <0.05 move on to Step 4
Step 4	Non-inferiority of automated AVR measurements from the left eye (where unavailable, measurements from the right eye will be used) against CBP for predicting hypertensive status in the untreated sample where automated AVR measurement is defined.

Hierarchical primary objectives each tested only if the preceding tests were significant.

Untreated patients are those who are not on any antihypertensive therapy at the time of ambulatory blood pressure monitoring.

The non-inferiority of retinal classification of hypertensive status compared with clinic blood pressure will be estimated using MedCalc and the R open source software based on one-sided Z-test. We will use a non-inferiority margin (Δ) on the AUC of 0.05. (This is the difference between the optimum method for combining multiple automated office blood pressure measurements, relative to ABPM, and a single blood pressure measurement [5].) , P-values will be determined with a Wald-test.

The hierarchical analysis will negate the need to adjust p values.

Secondary analysis:

Right eye AVR for predicting hypertensive status.

Average of left and right eye AVR for predicting hypertensive status.

Regression analysis of AVR with ABPM as a continuous variable.

Using night-time mean ABPM as reference standard instead of daytime ABPM.

Using 24-hr mean ABPM as reference standard instead of daytime ABPM.

AVR and demographic info (age, sex, BMI, medication) for predicting hypertensive status.

Clinic BP and demographic info (age, sex, BMI, medication) for predicting hypertensive status.

Totally automated analysis (with automated instead of AB testing for image quality).

Tables

Table 1: Study sample and group comparison

Table 2: Logistic regression and ROC analysis results

Figures:

ROC curves for each of the models run

Appendix

Assessment of artery vein ratio from scanning ophthalmoscope images.

During retinal imaging, most participants in RELIABLE had multiple images taken of the left eye and multiple images taken of the right eye. AB testing was used to select a single left and right eye for analysis. AB testing has been found to be an effective subjective method of ordering images in terms of quality. In the case of AB testing retinal images in RELIABLE, the original image was overlain with the vessel segmentation map so that both image and segmentation could be assessed. The AB testing was performed by an experienced researcher, where quality was assessed to maximise visible vasculature in measurement zone while minimising image artefacts such as eyelid and eyelash. This was performed as a pre-processing step before the automated and semi-automated artery vein ratio (AVR) analysis that is detailed below.

For each left and right eye pair per participant (where available), as identified by AB testing, the AVR was calculated. AVR was determined in an annular segment subtending 180˚ nasal to the optic disc and fixed on a line passing through the centre of the fovea and optic disc. It extended 6.5 to 8.5 optic disc radii from the optic disc centre.

Automated Method

AVR was evaluated in each ultra-widefield image as follows:

1. The optic disc location, and the fovea location were automatically detected [1]. These landmarks were used to establish the zone of measurement. A standard OD radius of 60 pixels was assumed.
2. Next, automatic segmentation of the retinal vessels was performed using a validated computer algorithm described elsewhere [2]. In brief, the green image was processed using scaled filters to increase vessel contrast with respect to the background. The filters exploited the morphology of the vessels as well as their cross-sectional intensity profile. A supervised machine learning classifier trained on a separate image set categorised image pixels as vessel or non-vessel, Figure 1 (d).
3. The calibre (in microns) for each vessel segment in the measurement zone was estimated as the average distance between parallel splines automatically fitted to the detected vessel edges [3]. Only vessel segments >= 26 pixels in length were measured. Segments below this length were discarded.
4. A deep learning model was used to perform classification of vessel segments as artery or vein (Optos; proprietary). In brief, centreline pixels from each vessel segment were extracted and patches of 129 x 129 pixels from contrast enhanced red green pseudo-colour images were passed to a CNN model. The output from the CNN model was a probability of each vessel segment centreline pixel being artery. The average probability of centreline pixels was found, and the vessel segment was classed as artery if the average probability was >= 0.5, otherwise it was classed as vein.
5. Finally, AVR was calculated as the ratio of the average calibre of the 3 widest arteries and the average of the 3 widest veins. Fewer artery and vein segments were used when less than 3 were available. Only vessel segments whose calibre was determined to be >= 0.06 mm and <= 0.25 mm were used for AVR calculation.

Semi-Automated Method

Note: please see [4] for recently published results of the semi-automated method.

AVR was evaluated in each ultra-widefield image as follows:

1. The optic disc location, its approximate outline, and the fovea location were manually annotated by a trained operator. These landmarks were used to establish the zone of measurement, Figure 1 (a).
2. Next, automatic segmentation of the retinal vessels was performed using a validated computer algorithm described elsewhere [2]. In brief, the green image was processed using scaled filters to increase vessel contrast with respect to the background. The filters exploited the morphology of the vessels as well as their cross-sectional intensity profile. A supervised machine learning classifier trained on a separate image set categorised image pixels as vessel or non-vessel, Figure 1 (d).
3. The calibre (in microns) for each vessel segment in the measurement zone was estimated as the average distance between parallel splines automatically fitted to the detected vessel edges [3]. Only vessel segments >= 26 pixels in length were measured. Segments below this length were discarded.
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5. The retinal vessels detected in the zone of measurement were overlaid on the original image. The operator labelled false positives in the detected vasculature (eyelashes, choroidal vessels etc.) and corrected misclassified artery and vein vessel segments according to their appearance and connectivity.

Finally, AVR was calculated as the ratio of the average calibre of the 3 widest arteries and the average of the 3 widest veins. Fewer artery and vein segments were used when less than 3 were available. Only vessel segments whose calibre was determined to be >= 0.06 mm and <= 0.25 mm were used for AVR calculation.

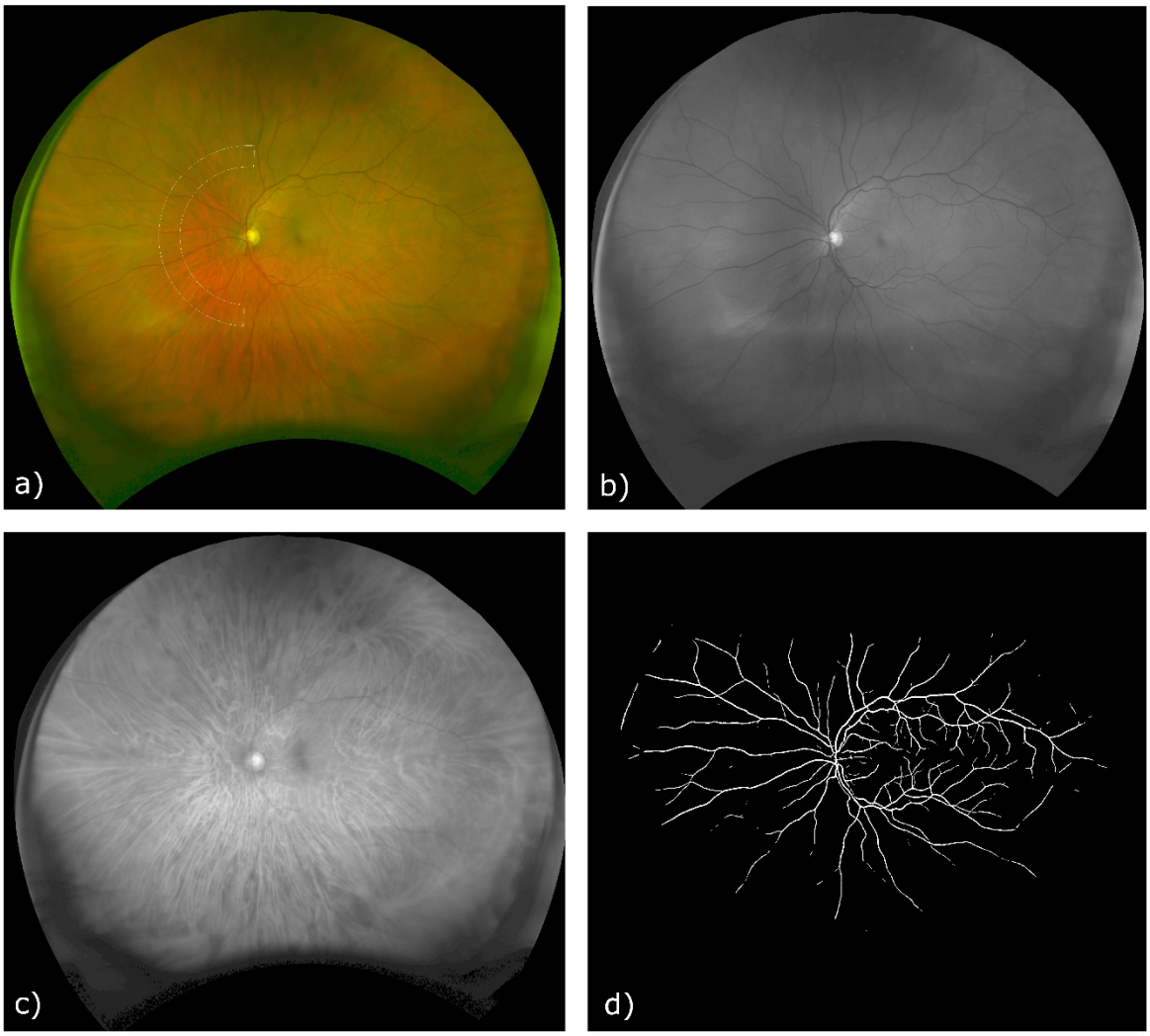

Figure 1 : (a) Pseudo-colour image of the retina showing a region of interest, at a distance of 6.5–8.5 optic disc radii from the optic disc centre, in which artery vein ratio was calculated. (b) Green laser (532 nm) image showing the retinal vasculature contrasted against the retinal surface. (c) Red laser (633 nm) image showing choroidal vessels as well as some of the larger surface venules. (d) Automatically generated retinal vascular map.

References:

[1]: Wakeford PR, Pellegrini E, Robertson G, et al. Optic Disc and Fovea Localisation in Ultra-widefield Scanning Laser Ophthalmoscope Images Captured in Multiple Modalities. In: Zheng Y, Williams BM, Chen K, eds. Medical Image Understanding and Analysis. Cham: : Springer International Publishing 2020. 399–410. doi:10.1007/978-3-030-39343-4_34

[2]: Pellegrini E, Robertson G, Trucco E, et al. Blood vessel segmentation and width estimation in ultra-wide field scanning laser ophthalmoscopy. Biomed Opt Express 2014;5:4329.

[3]: Cavinato A, Ballerini L, Trucco E, et al. Spline-based refinement of vessel contours in fundus retinal images for width estimation. Proc - Int Symp Biomed Imaging 2013:872–5.

[4]: Robertson G, Fleming A, Williams MC on behalf of Northern Ireland Cohort of Longitudinal Ageing, et al. Association between hypertension and retinal vascular features in ultra-widefield fundus imaging. Open Heart 2020;7:e001124. doi: 10.1136/openhrt-2019-001124

[5] Myles N Moore, Martin G Schultz, Mark R Nelson, J Andrew Black, Nathan B Dwyer, Ella Hoban, Matthew D Jose, Wojciech Kosmala, Monika Przewlocka-Kosmala, Jowita Zachwyc, Petr Otahal, Dean S Picone, Philip Roberts-Thomson, Panagiota Veloudi, James E Sharman, Identification of the Optimal Protocol for Automated Office Blood Pressure Measurement Among Patients With Treated Hypertension, *American Journal of Hypertension*, Volume 31, Issue 3, March 2018, Pages 299–304, https://doi.org/10.1093/ajh/hpx180

Checksums

Repository	**Git commit checksum**
Auto a (data files for outer measurement area)	7a0569f7d0866a6a7f0ff8d796e8292029609e68
Auto b (data files for inner measurement area)	875cda3a0c47207d11dcde93e8703911604a5f76
Semi a (data files for outer measurement area)	e8c08162c23d051e74b7c2c1193cb1d4c4432a06
Semi b (data files for inner measurement area)	c7628d80bc852c349d7e35b4f8c79eabd81da477
Ab testing: code for performing AB testing	458c9881691b76502e3e0ccddd90bb19986da765
Annotation (GUI for manual correction)	9b33115fad2c3b4ecc2342620ce4ec6607ca2d29
Auto (Auto analysis code)	2f291c84fc61aff7babe90ae1dbff27938d08f49
Avr (Semi automated analysis code)	37d281df2c925a75b545aab35ce79db5cbc2179b
Odfovea (manual OD and fovea annotation)	8134f48ada6d3bead4faf23846375eb753691117
Projection (Stereographic projection code)	983415ece2b38f1559817a35f4825d0774e46211
Scripts (code)	c8ac3f9a12ddd80e32f989eb6e5e53c58aff15d9
Sort (code)	868fd71cb355e4a480a172f360485f2629f32bdd
Results for stats (excel sheets with results for Sarah)	c3cf652e960f4dba5210c2f81bd892fd09dd9ccd
Odfovea auto (Automated OD and fovea detection)	2fec5fb0c2962695f5c8df11e1e9e9f89ab3e9da
Filename	**SHA-256 Checksum**
reliable_git_repo.zip (a zip folder containing the above git repos minus data files)	bb0c6f519e8a315039576a2fdc61525c3c94951f2628ec0a5d0d8e08c89f65fd
reliable_results_a.zip (a zip folder containing the outer area data files)	eaf7fca57db2e04a53b2d5e84d499a584ea57937ed293ab53a10cf04b2064f0e
reliable_results_b.zip (a zip folder containing the inner area data files)	ca4a4a3b4533a33cf01368b64dd7b4790cec505253aba5da394eede50d1f8eae