Clinically valid conclusions from retinal photographs need the best formulae

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Cardiovascular and cerebrovascular diseases are common causes of morbidity and mortality. Modifiable risk factors such as tobacco smoking, a diet high in saturated fatty acids, physical inactivity, high cholesterol level, hypertension, and a high body mass index are all associated with retinal vascular changes [1]. The retinal superficial vasculature offers a unique possibility to noninvasively and directly observe the microvasculature and, through photographic imaging enables quantitative measuring of the retinal vessels with high reproducibility [1].

Changes in the ocular circulation due to systemic disease may lead to loss of vision due to a lack of perfusion. Observation of the retinal microcirculation may therefore allow the detection of early changes and also serve as a marker to track any changes over time, to assess changes in lifestyle and treatment efficacy.

Arterial walls grow thicker during the decades leading to atherosclerosis. Differentiation between mechanisms of action needs physiologically sensible measurements. Central retinal artery caliber is inferred from arteriolar diameters measured on photos. Formulae for such central retinal artery equivalent (CRAE) were developed by Parr, Hubbard, and Knudtson [1–3]. As blood pressure should be an almost linear function of the ratio of arterial and venular caliber ($AVR = CRAE / CRVE$) [1], the central retinal vein equivalent (CRVE) was defined similarly, adjusting for thinner walls.

Blood flow is proportional to the lumen of vessels rather than caliber. Therefore, CRAE and CRVE internally average 2 lumina, before converting back to the caliber. The

calculation is repeated for the results to mimic bifurcation. The Parr, Hubbard, and Knudtson formulae can be thought of as weighted means with lower weights for the largest and the smallest calibers measured. If an uneven number of measurements is used, the median gets a higher weight. If an even number of measurements is used, either the limits of the median class get the higher weight, or more extreme measurements. In the case of 4 measurements, even the most extreme values could get the highest weight. The algorithm may thus be robust (5 measurements) or may propagate variability of outliers (4 measurements). CRAE, unweighted average of calibers and root of mean lumen result in different numbers, may not be compared, but they are highly correlated as long as the same measurements are used. Using different numbers of measurements drastically reduces the association.

The number of arterioles and venules measured can bias CRAE and CRVE [3]. Choosing the four thickest vessels should result in a greater mean than choosing the six thickest vessels, i.e. considering two thinner ones, too. So, the number of vessels measured should be constant within a study for internal validity. It should be the same as in other studies for external validity.

Measurements of blood vessel calibers should all be made in the same region. The standard region would be shifted, if the axial length is disregarded. A recent publication highlights the importance of measurement zone [4]. Figure 1 illustrates the increase of the measurement zone in longer eyes and the additional change in relative location. This shift in size and location impacts all ocular structures, as the corresponding measurement points of the retinal nerve fiber layer (RNFL) can no longer be directly compared to the normative database. The shift in location has therefore relocated the measurement point to a different retinal location and as a consequence, a lower or higher than normal measurement maybe obtained, but still leaving the observer in the dark about the true thickness. This also applies to measurement zones defined for retinal vessel caliber measurements and caliber size. However, while the measurement zone in a longer eye becomes enlarged (as in covering a larger area), the opposite applies to the detail

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Fig. 1 Shift of measurement region (double arrows) by axial length. Note the increased distance from the optic disc

within the measurement zone, meaning that while a larger area is assessed, the vessel diameter within this area would be smaller than its value at the correct location.

Reference to the disc diameter is recommended. This varies between eyes. Not all measurement systems account for individual disk sizes. Adjustments for two-dimensional or three-dimensional space are more complex than adjustments for a single dimension.

Ocular magnification differs from eye to eye depending on its length and refractive power, so that the size of structures in the retinal plane may need to be corrected. To do so, a factor must be applied [5]. However, the formulae available to correct for ocular magnification often require the input of axial length of the individual eye and often only apply for a particular camera design [5, 6]. Correction/adjustment cannot be achieved by including refraction only as a covariate in linear regression, as that would just add something where a multiplication is needed. This approximation of a curve by a straight line may be good enough, if eyes with very similar refraction and axial length are investigated. A better way to deal with this source of bias would be to correct measurements for ocular magnification upfront, rather for axial length than just refraction.

When drawing conclusions about vessel calibers and their link with other variables or to define vessel narrowing should only be conducted when the calculation of vessel calibers was conducted using a standardized approach.

A standardized approach to calculate retinal vessel calibers was introduced to overcome the subjective and unreliable nature of visual observation by ophthalmoscope [7]. Many of the proposed visual grading scales (Keith-Waggoner-Barker, Scheie) suggested the arterio-venous-ratio (AVR) as a measure to quantify arteriolar narrowing. When moving to more quantitative methods, some studies explicitly measured arteriolar and venular widths as measured from projected images.

While such a ratio has the advantage to quantify the relation of artery to vein diameters, it also provides some limited adjustment with respect to vessel caliber size (i.e. individuals with narrower arteries tend to have correspondingly narrower veins, in the absence of hypertension and diabetes mellitus). Using a ratio rather than absolute vessel widths also accounts for ocular magnification due to refractive error and axial length as it affects arteries and veins with the same magnification factor. Apart from these minor improvements, such a ratio measurement (a) cannot tell the difference between pathological changes which may occur only on the arteriolar or venular side and (b) may overlook changes occurring simultaneously with the same magnitude, which would lead to an unchanged AVR. While these early studies showed a link between increased blood pressure and reduced vessel caliber, there was a need to improve its reproducibility and reliability as well as to the introduction of a more “automated” measurement approach for it to become clinically useful.

Subsequent research by Parr, Hubbard, and Knudtson [1–3] introduced a standardized measurement zone and formulae, which account not only for branching pattern but also for the vessel geometry of the vasculature in that the square of the individual diameters is used for vessel caliber calculation.

Today, there is a range of different software platforms available to extract CRAE, CRVE, and AVR in a semiautomatic fashion. Measurement equipment and algorithm used in one patient or one study should, however, be kept constant. Or some correction has to be applied. Saved images could be analyzed uniformly ex post.

To allow successful tracking of changes over time and to allow study comparability, it is therefore paramount to adhere to a standard protocol. Besides study comparability, this would also help establish cut-off values for clinical purposes in the future. While imaging hardware and software will no doubt further improve in the future, there will always remain some issues which will contribute to the overall data variability, such as illumination, contrast, ocular media transparency, pulse, and others. As we are unable to control all factors, it is even more important to standardize those which we can control with ease such as the measurement zone, magnification, vessel inclusion, and formulae used for calculation.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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